Exhibit C

Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

Research Article

Cancer Prevention Research

Genital Powder Use and Risk of Ovarian Cancer: A Pooled Analysis of 8,525 Cases and 9,859 Controls

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Abstract

Genital powder use has been associated with risk of epithelial ovarian cancer in some, but not all, epidemiologic investigations, possibly reflecting the carcinogenic effects of talc particles found in most of these products. Whether risk increases with number of genital powder applications and for all histologic types of ovarian cancer also remains uncertain. Therefore, we estimated the association between self-reported genital powder use and epithelial ovarian cancer risk in eight population-based case control studies. Individual data from each study were collected and harmonized. Lifetime number of genital powder applications was estimated from duration and frequency of use. Pooled ORs were calculated using conditional logistic regression matched on study and age and adjusted for potential confounders. Subtype-specific risks were estimated according to tumor behavior and histology. 8,525 cases and 9,859 controls were included in the analyses. Genital powder use was associated with a modest increased risk of epithelial ovarian cancer [OR, 1.24; 95% confidence interval (CI), 1.15–1.33] relative to women who never used powder. Risk was elevated for invasive serous (OR, 1.20; 95% CI, 1.09 1.32), endometrioid (OR, 1.22; 95% CI, 1.04 1.43), and clear cell (OR, 1.24; 95% CI, 1.01 1.52) tumors, and for borderline serous tumors (OR, 1.46; 95% CI, 1.24–1.72). Among genital powder users, we observed no significant trend ($P = 0.17$) in risk with increasing number of lifetime applications (assessed in quartiles). We noted no increase in risk among women who only reported nongenital powder use. In summary, genital powder use is a modifiable exposure associated with small-to-moderate increases in risk of most histologic subtypes of epithelial ovarian cancer. *Cancer Prev Res; 6(8); 811–21. ©2013 AACR.*

Introduction

Powders that are commonly applied either directly to the genital, perineal, or rectal area after bathing or indirectly to underwear, sanitary napkins, tampons, or stored contraceptive devices may contain talc because of its softness, absorbency, and lack of clumpiness (1). However, the presence of talc in commercially available powder formulations has varied over time, even within particular brands of products, limiting the ability of most epidemiologic studies to measure genital talc exposure accurately. Despite this, genital powder use, but not use on other parts of the body, has been linked to increased risk of ovarian cancer, suggesting that powder particles ascending the genital tract may predispose to ovarian cancer development (2 4). Meta-analyses of

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doi: 10.1158/1940-6207.CAPR-13-0037

Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

observational studies show 33% to 35% increased risk of ovarian cancer among women who have used genital powders (1, 4, 5), but evidence for a dose response relationship has been inconsistent. Although dose-response was not addressed in previous meta-analyses (1, 4, 5), some individual studies have reported significant dose-response (4, 6 10) while others have not (9, 11 15).

Epidemiologic and biologic studies show differences in risk-factor profiles and molecular characteristics between ovarian cancer subtypes defined by histology (serous, endometrioid, mucinous, and clear cell) and behavior (borderline and invasive; refs. 16 and 17). For instance, serous tumors are characterized by p53 mutations, whereas mucinous tumors have a high prevalence of KRAS mutations (17) and are not generally associated with reproductive risk factors (16, 18). Because most early studies of powder use and ovarian cancer did not include analysis by histologic subgroups (3, 6, 11, 19–21), histology-specific estimates were not available from these studies for meta-analysis. Most (2, 4, 8, 9, 22), but not all (10, 14, 15, 23), epidemiologic studies of genital powder use and risk of ovarian cancer that have evaluated histologic subgroups have found the association to be strongest for serous-invasive tumors. Such tumors comprise the most common variety of ovarian cancer and few previous studies have had sufficient statistical power to evaluate the association between genital powder use and risk of other histologic subtypes. In the present study, we evaluated associations between genital powder use and risk of ovarian cancer overall, by invasiveness and by histologic type in a pooled analysis of eight population-based case control studies with relevant data from the Ovarian Cancer Association Consortium (OCAC), a consortium founded in 2005 to validate promising genetic associations in epidemiologic studies of ovarian cancer.

Materials and Methods

Participating studies

Studies participating in the OCAC consortium as of April 2010 that collected data on powder use were included. Each study was approved by an institutional ethics committee and all participants provided informed consent. Detailed description of the OCAC consortium is available elsewhere (24). Characteristics of the eight case control studies contributing data to this analysis are presented in Table 1. Six studies were conducted in the United States [Diseases of the Ovary and their Evaluation Study (DOV; ref. 14), Hawaii Ovarian Cancer Study (HAW; ref. 25), Hormones and Ovarian Cancer Prediction Study (HOP; ref. 26), North Carolina Ovarian Cancer Study (NCO; ref. 27), New England Case Control Study of Ovarian Cancer (NEC; ref. 4), and University of Southern California Study of Lifestyle and Women s Health (USC; ref. 28)], one study in Australia [Australian Cancer Study (AUS; ref. 7)], and one study in Canada [Southern Ontario Ovarian Cancer Study (SON; ref. 15)]. Overall, our analyses included 8,525 cases of ovarian, fallopian tube, or peritoneal cancer and 9,859 controls. Five studies previously reported on powder use [AUS (7), DOV (14), NCO (27), NEC (4), and SON (15)], three of which provided data for this analysis that had not been included in their previous powder-related publication (DOV, NEC, and AUS). The remaining three studies have not previously published their genital powder use data (HAW, HOP, and USC).

Exposure and covariate data

Data collected from participants about genital powder use varied between studies. Harmonized analytic exposure variables were developed by comparing questionnaires between the eight participating studies. The majority of the studies have obtained information on duration and frequency of powder use, age at first powder use, use by sexual partners, and non-genital use (Table 1). We defined genital powder use as any type of powder (talc, baby, deodorizing, cornstarch, or unspecified/unknown) applied directly or indirectly (by application to sanitary pads, tampons, or underwear) to the genital, perineal, or rectal area. Because study-specific powder questions included varying degrees of detail about type and method of application, genital powder definitions differ between studies. Criteria for regular genital powder use varied between studies from "ever use" (AUS) to "one year or longer" (DOV); the specific wording for this question is provided in Table 1. Use of body powders on sites other than the genital area was defined as non-genital powder use. Women who reported both genital and non-genital powder use were classified as genital users. Two studies (DOV and SON) did not collect data on nongenital use, and therefore women assigned to "no powder use" for these studies could have a history of non-genital powder exposure. Extensive information on known and suspected risk factors for ovarian cancer was collected in each study, including oral contraceptive use, parity, tubal ligation history, body mass index (BMI), race, and ethnicity.

Statistical analysis

Participants missing case/control status ($n = 17$) or tumor histology ($n = 19$) were excluded from the analysis. We also excluded 1,119 participants who answered "do not know" or were missing data on genital powder use; most of these were from the NCO study, which did not include genital powder questions for the first 720 participants. Furthermore, we excluded participants missing tubal ligation ($n = 55$), oral contraceptive duration ($n = 100$), parity ($n = 3$), or height or weight (BMI; $n = 179$). To examine differences in characteristics between cases and controls, we evaluated two-sample t statistics (age and BMI) and χ^2 statistics (oral contraceptive use, nulliparity, tubal ligation, race/ethnicity, and powder use).

Study-specific ORs and 95% confidence intervals (CI) were estimated using unconditional logistic regression and were summarized by forest plots, including study heterogeneity based on Cochran s Q statistic. As no significant heterogeneity was observed between studies, we calculated pooled ORs and 95% CIs across the studies using conditional logistic regression matched on 5-year age groups and

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Table 1. Characteristics of eight studies included in the analysis of genital powder use and ovarian cancer

Study[a]	Diagnosis years	Controls	Cases	Histology[b]				Behavior[c]		Question used to define genital powder use
				Serous	Mucinous	Endometrioid	Clear cell	Invasive	Borderline	
AUS[9]	2002–2006	1,449	1,432	889 (62%)	174 (12%)	132 (9%)	78 (5%)	1,158 (81%)	274 (19%)	Have you ever used any sort of powder or talc on your genital area, in your underwear or on a sanitary pad or diaphragm?
DOV[9]	2002–2009	1,841	1,565	905 (58%)	186 (12%)	201 (13%)	87 (6%)	1,153 (74%)	412 (26%)	Before (reference date) did you ever use any of the following products routinely during 1 month or more? Powder on sanitary napkins or pads? Vaginal deodorant spray? Before (reference date) did you usually apply any powder to your genital (perineal) area after bathing? We are only interested in times when you did this for at least 1 year or longer.[d]
HAW	1993–2008	755	481	222 (46%)	87 (18%)	69 (14%)	47 (10%)	392 (82%)	89 (19%)	Before (month/year of diagnosis[e]) did you ever use talc, baby, or deodorizing powder dusted or sprayed on your body? By regularly I mean at least once a month for 6 months or more. Did you ever use talc, baby, or deodorizing powder as a dusting powder to the genital or rectal area? As a dusting powder to sanitary napkins? As a dusting powder to underwear? On a diaphragm or cervical cap?
HOP	2003–2008	1,489	735	433 (59%)	53 (7%)	75 (10%)	47 (6%)	568 (88%)	80 (12%)	As an adult and before (reference month/year) did you ever use talc or baby powder or deodorizing powder with talc at least once a month for 6 months or more in any of the following ways: as a dusting powder or deodorizing spray to your genital or rectal areas? On your sanitary napkin? On your underwear? On your diaphragm or cervical cap?
NCO[9]	1999–2008	650	786	489 (62%)	71 (9%)	100 (13%)	65 (8%)	636 (81%)	148 (19%)	Did you ever regularly use cornstarch, talc, baby, or deodorizing powders (dusted or sprayed) at least 1 time per month for at least 6 months? If yes, please tell me if you used cornstarch, talc, baby, or deodorizing powders in any of the following ways: directly to your genital or rectal areas? Applied to your sanitary napkins or tampons? Applied to birth control devices such as cervical cap or diaphragm? Applied to your underwear?

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Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

Table 1. Characteristics of eight studies included in the analysis of genital powder use and ovarian cancer (Cont'd)

Study[a]	Diagnosis years	Controls	Cases	Histology[b]				Behavior[c]		Question used to define genital powder use
				Serous	Mucinous	Endometrioid	Clear cell	Invasive	Borderline	
NEC[g]	1992–2008	2,329	2,305	1,234 (54%)	281 (12%)	352 (15%)	276 (12%)	1,659 (77%)	486 (23%)	Did you ever regularly use powder on your body or your underwear (at least once per month for any amount of time)? If yes, did you apply powder directly to your genital or rectal areas? To your sanitary napkins or tampons? To your underwear?[f]
SON[g]	1989–1992	564	449	254 (57%)	80 (18%)	71 (16%)	29 (6%)	365 (81%)	84 (19%)	Have you ever used sanitary napkins/tampons? If yes, could you tell me over what ages you have used them, for how many years, what percentage of periods you have used them for, the usual number you have used for each period, whether they were deodorant pads/tampons, and if you used talcum powder or starch on them? Have you ever regularly used talcum powder or starch on your vaginal area after showering or bathing?
USC	1993–1997	782	772	396 (52%)	131 (17%)	75 (10%)	32 (4%)	549 (73%)	205 (27%)	Before (reference month/year), did you ever regularly use talc, baby, or deodorizing powder dusted or sprayed on your body? By regularly I mean at least once a month for 6 months or more. Did you ever use talc, baby, or deodorizing powder as a dusting powder to the genital or rectal area? As a dusting powder to sanitary napkins? As a dusting powder to underwear? On a diaphragm or cervical cap?

[a]AUS, Australian Cancer Study; DOV, Diseases of the Ovary and their Evaluation Study; HAW, Hawaii Ovarian Cancer Study; HOP, Hormones and Ovarian Cancer Prediction Study; NCO, North Carolina Ovarian Cancer Study; NEC, New England Case Control Study; SON, Southern Ontario Ovarian Cancer Study; and USC, University of Southern California Study of Lifestyle and Women's Health.

[b]Cases listed by histology do not sum because mixed, other, undifferentiated, and unknown are not included.

[c]Cases listed by behavior do not sum to the total number of cases because 267 cases are missing behavior information.

[d]In a separate series of questions, participants were asked about powder use with diaphragm storage. Duration was calculated from ages of use. Information on duration, frequency, and timing of use was only collected on genital/perinal powder use after bathing.

[e]Controls were asked "Have you ever regularly used. . ."

[f]NEC question varied slightly between the three study phases. Between 1992 and 1997 participants were asked, "As an adult and before (reference month/year), did you regularly use talc, baby, or deodorizing powders dusted or sprayed to your body in any of the following ways:". Between 1998 and 2003, women were asked "Did you regularly apply cornstarch, talc, baby, or deodorizing body powder at least one time per month for 6 months or longer? If yes, please tell me if you regularly applied cornstarch, talc, baby or deodorizing body powders in any of the following ways:" Between 2003 and 2008 participants were asked the question listed above.

[g]These studies previously published on genital powder use and ovarian cancer risk. AUS, DOV, and NEC provided new data to the pooled analyses presented here that were not included in previous publications.

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study. All analyses were adjusted for potential confounders: age (continuous), duration of oral contraceptive use (never use, use <2, 2 <5, 5 <10, or 10 years), parity (0, 1, 2, 3, or 4 children), tubal ligation history, BMI (quartiles based on distribution in controls), and race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other). Family history of breast or ovarian cancer was also considered as covariate but was not included in the final model.

Subtype-specific estimates were calculated for subgroups of ovarian cancer defined by behavior (invasive and borderline) and histology (serous, mucinous, endometrioid, and clear cell) by comparing each case group with all controls. As borderline endometrioid and clear cell tumors are rare, we did not have sufficient numbers to evaluate those types separately.

To measure cumulative dose of genital powder use, we estimated lifetime number of powder applications by multiplying total months of use by frequency of use per month, for all direct and indirect genital powder applications. Women who reported multiple types of genital powder exposure (on underwear, on sanitary napkins or pads, or directly to genital area) during the same time period were assigned the number of genital powder applications equal to the most commonly used type rather than the sum of applications across all types of genital powder exposure. We reasoned that contemporaneous powder applications were unlikely to be independent events and therefore should not be treated cumulatively. Analyses of estimated lifetime number of applications excluded participants in the HOP study as data on age and frequency of use were not collected ($n = 2{,}224$); genital powder users missing information on duration or frequency of use were omitted in the remaining studies ($n = 394$). Never-regular users of genital powders and women who only reported nongenital use were coded as having zero lifetime genital powder applications and comprised the reference group for this analysis. Categories were determined on the basis of age-specific quartile cutoff points in controls (25th, 50th, and 75th percentile cutoff points are 612, 1,872, and 5,400 for participants < 40 years old; 612, 2,160, and 7,200 for 41 50 years; 720, 3,600, and 10,800 for 51 60 years; 1,440, 5,760, and 14,440 for 61 70; 840, 7,200, and 18,000 for > 70 years). Trends were evaluated on the basis of the median lifetime number of genital powder applications for controls in each age-specific quartile using the Wald statistic and were conducted both including and excluding never users of genital powders.

We estimated the association between genital powder use and ovarian cancer risk within strata to evaluate potential modification of effect defined using a cutoff point BMI of 30 based on the World Health Organization's definition of obesity, endometriosis, parity, tubal ligation/hysterectomy, and menopausal status. We used likelihood-ratio statistics comparing models with and without interaction terms to determine statistically significant interactions. To estimate calendar year of first use, we subtracted the years since first use (age at study entry minus age at first genital powder use) from median calendar year of the participant s study.

All analyses were conducted in SAS v9.2 (SAS) and Stata v9.2 (StataCorp). All *P* values are two-sided. Analyses have been independently verified by two separate study groups (HAW and NCO).

Results

This pooled analysis of eight case control studies included 9,859 controls and 8,525 ovarian cancer cases. Genital powder use was reported by 2,511 (25%) of the controls and 2,600 (31%) of the cases, whereas powder use only on other (nongenital) parts of the body was reported by 1,533 (16%) of the controls and 1,282 (15%) of the cases (Table 2). The prevalence of genital powder use in controls varied widely between study sites, highest in AUS (45%) and lowest in HAW (15%; Table 3).

In the pooled analysis, ever-regular use of genital powder was associated with a modest increase in risk of ovarian cancer (OR, 1.24; 95% CI, 1.15 1.33; Table 3) relative to women who reported no powder use (AUS, HAW, HOP,

Table 2. Characteristics of cases and controls included in the pooled analysis[a]

	Controls ($N = 9{,}859$) Mean (STD) or *N* (%)	Cases ($N = 8{,}525$) Mean (STD) or *N* (%)
Age	55 (12)	55 (12)
Oral contraceptive use		
Never	2,995 (30)	3,411 (40)
Ever	6,864 (70)	5,114 (60)
Parous		
No	1,468 (15)	2,196 (26)
Yes	8,391 (85)	6,329 (74)
Tubal ligation		
No	7,359 (75)	6,994 (82)
Yes	2,500 (25)	1,531 (18)
BMI	26.5 (6.1)	27.0 (6.6)
Race/ethnicity		
Non-Hispanic White	8,629 (88)	7,433 (87)
Hispanic White	197 (2)	214 (3)
Black	273 (3)	268 (3)
Asian	350 (4)	313 (4)
Other[b]	407 (4)	291 (4)
Powder use[c]		
Never use	5,815 (59)	4,643 (54)
Non-genital use only	1,533 (16)	1,282 (15)
Genital use	2,511 (25)	2,600 (31)

[a]All characteristics listed except age differed significantly (<0.01) between cases and controls. Cases include both borderline and invasive ovarian cancers.
[b]There are 6 cases and 3 controls missing race/ethnicity information.
[c]Categories for non-genital and genital powder use are mutually exclusive.

Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

Table 3. Association between powder use and risk of ovarian cancer (borderline and invasive combined) by study site

Site	Controls (%) ($N = 9,859$)	Cases (%) ($N = 8,525$)	Age-adjusted OR (95% CI)[a]	Multivariate OR (95% CI)[a]
AUS				
No powder use	305 (21)	300 (21)	1.00	1.00
Non-genital use only	486 (34)	427 (30)	0.85 (0.69–1.05)	0.92 (0.74–1.14)
Genital use	658 (45)	705 (49)	1.04 (0.85–1.26)	1.13 (0.92–1.38)
DOV[b]				
No powder use	1,544 (83)	1,293 (83)	1.00	1.00
Genital use	297 (16)	272 (17)	1.14 (0.95–1.37)	1.13 (0.93–1.36)
HAW				
No powder use	489 (65)	326 (68)	1.00	1.00
Non-genital use only	154 (20)	81 (17)	0.79 (0.58–1.07)	0.69 (0.50–0.96)
Genital use	112 (15)	74 (15)	0.99 (0.72–1.37)	0.99 (0.70–1.41)
HOP				
No powder use	989 (66)	439 (60)	1.00	1.00
Non-genital use only	184 (13)	102 (14)	1.23 (0.94–1.61)	1.23 (0.93–1.62)
Genital use	316 (21)	194 (26)	1.37 (1.11–1.69)	1.34 (1.07–1.67)
NCO				
No powder use	391 (60)	469 (60)	1.00	1.00
Non-genital use only	137 (21)	122 (16)	0.75 (0.57–0.99)	0.74 (0.56–0.99)
Genital use	122 (19)	195 (25)	1.33 (1.03–1.74)	1.37 (1.05–1.80)
NEC				
No powder use	1,239 (53)	1,129 (49)	1.00	1.00
Non-genital use only	454 (19)	421 (18)	1.02 (0.87–1.19)	1.04 (0.88–1.22)
Genital use	636 (27)	755 (33)	1.30 (1.14–1.49)	1.28 (1.12–1.47)
SON[b]				
No powder use	364 (65)	252 (56)	1.00	1.00
Genital use	200 (35)	197 (44)	1.43 (1.11–1.85)	1.35 (1.03–1.76)
USC				
No powder use	494 (63)	435 (56)	1.00	1.00
Non-genital use only	118 (15)	129 (17)	1.25 (0.94–1.66)	1.14 (0.85–1.52)
Genital use	170 (22)	208 (27)	1.39 (1.10–1.77)	1.36 (1.06–1.74)
Pooled[c]				
No powder use	5,815 (59)	4,643 (54)	1.00	1.00
Non-genital use only	1,533 (16)	1,282 (15)	0.98 (0.90–1.07)	0.98 (0.89–1.07)
Genital use	2,511 (25)	2,600 (31)	1.25 (1.16–1.34)	1.24 (1.15–1.33)

[a]Study-specific estimates were determined using unconditional logistic regression and pooled ORs were estimated using conditional logistic regression conditioned on 5-year age groups and study. Multivariate models are adjusted for age (continuous), oral contraceptive duration (never use, <2, 2–<5, 5–<10, or 10 years), parity (0, 1, 2, 3, or 4+ children), tubal ligation history (no or yes), BMI (quartiles), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other).

[b]Information on non-genital powder use was not collected in the SON and DOV study.

[c]*P* value for heterogeneity between multivariate study specific ORs equal to 0.61; calculated using Conchran's *Q* statistic test.

NCO, NEC, and USC) or no genital powder use (DOV and SON). We observed no heterogeneity in the risk associated with genital powder use between studies regardless of the reference group ($P = 0.61$; Fig. 1). Results were similar for genital powder users compared with a combined reference group including never users and women whose use of powder was exclusively non-genital (covariate-adjusted OR, 1.25; 95% CI, 1.16–1.34; data not shown), reflecting the absence of an association between powder use on other parts of the body with ovarian cancer risk (Table 3).

Genital powder use was associated with a similar increased risk of borderline and invasive ovarian cancer overall (Table 4). For borderline tumors, the association was stronger for the serous subtype (OR, 1.46; 95% CI, 1.24–1.72; Table 4) and nonsignificant for the mucinous subtype. For invasive ovarian cancer, we observed small

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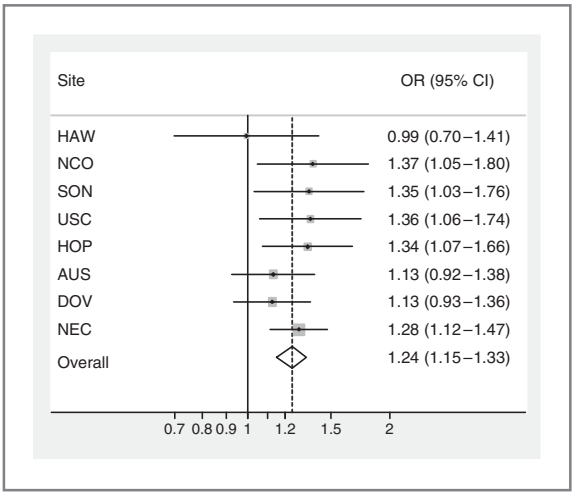

Figure 1. Association between genital powder use and ovarian cancer risk in eight studies, $P_{heterogeneity} = 0.61$. Adjusted for age (continuous), oral contraceptive duration (never use, <2, 2–<5, 5–<10, or 10 years), parity (0, 1, 2, 3, or 4+ children), tubal ligation history, BMI (quartiles), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other) and non-genital powder use. Studies listed in decreasing order of effect size SE (funnel plot). No evidence of heterogeneity based on Conchran's Q statistic ($P = 0.61$).

increases in risk of serous (OR, 1.20; 95% CI, 1.09 1.32), endometrioid (OR, 1.22; 95% CI, 1.04 1.43), and clear cell (OR, 1.24; 95% CI, 1.01–1.52) cancer but no significant increase in risk of mucinous cancer (OR, 1.09; 95% CI, 0.84–1.42). Similarly, we observed no significant increase in risk when borderline and invasive mucinous tumors were considered together (data not shown). Risk associated with genital powder use was consistent across studies for borderline and invasive tumors as well as invasive serous, endometrioid, and clear cell subtypes ($P_{heterogeneity} > 0.1$; Fig. 2A E), but not for mucinous tumors ($P = 0.08$; Fig. 2F). Genital powder use was associated with increased risk of invasive mucinous tumors in SON, HOP (significantly), and USC (nonsignificantly), whereas in the remaining studies (HAW, NCO, AUS, DOV, and NEC) genital powder use was nonsignificantly associated with reduced risk.

We evaluated cumulative genital powder exposure as a composite variable of frequency and duration of use. We observed similar increased risks of all nonmucinous subtypes of epithelial ovarian cancer combined across quartiles of genital powder compared with nonuse: OR_{Q1}, 1.18; 95% CI, 1.02 1.36; OR_{Q2}, 1.22; 95% CI, 1.06 1.41; OR_{Q3}, 1.22; 95% CI, 1.06 1.40; OR_{Q4}, 1.37; 95% CI, 1.19 1.58 (Table 5). Although a significant increase in risk with an increasing number of genital powder applications was found for nonmucinous epithelial ovarian cancer when nonusers were included in the analysis ($P_{trend} < 0.0001$), no trend in cumulative use was evident in analyses restricted to ever-users of genital powder ($P_{trend} = 0.17$; Table 5). Taken together, these observations suggest that the significant trend test largely reflects the comparison of ever-regular use with never use. Because tubal ligation or hysterectomy would block the transport of powder through the genital tract to the ovaries, we conducted a sensitivity analysis excluding women who started genital powder use after these procedures. We observed similar associations when

Table 4. Association between powder use and risk of ovarian cancer by behavior and histology

	Model 1[a]			Model 2[a]		
	No powder use	Genital powder use		No genital powder use	Genital powder use	
	n (%)	*n* (%)	OR (95% CI)[b]	*n* (%)	*n* (%)	OR (95% CI)[b]
Controls	5,815 (59)	2,511 (25)		7,348 (75)	2,511 (25)	
All borderline cases	1,035 (58)	504 (28)	1.29 (1.14–1.48)	1,247 (72)	504 (28)	1.30 (1.15–1.47)
Serous	567 (57)	300 (30)	1.46 (1.24–1.72)	700 (70)	300 (30)	1.45 (1.24–1.69)
Mucinous	409 (60)	184 (27)	1.17 (0.96–1.42)	502 (73)	184 (27)	1.19 (0.98–1.43)
All invasive cases	3,470 (54)	2,009 (31)	1.21 (1.12–1.32)	4,471 (69)	2,009 (31)	1.23 (1.14–1.32)
Serous	1,952 (53)	1,197 (32)	1.20 (1.09–1.32)	2,519 (68)	1,197 (32)	1.24 (1.13–1.35)
Mucinous	206 (57)	94 (26)	1.09 (0.84–1.42)	269 (74)	94 (26)	1.06 (0.82–1.36)
Endometrioid	568 (55)	304 (30)	1.22 (1.04–1.43)	723 (70)	304 (30)	1.20 (1.03–1.40)
Clear Cell	327 (54)	187 (31)	1.24 (1.01–1.52)	420 (69)	187 (31)	1.26 (1.04–1.52)

[a]In model 1, the reference group is restricted to women with no powder use except for the DOV and SON studies as these did not collect data on non-genital powder use. The number of cases who reported non-genital powder use was 212 (13%) of all borderline cases, 133 (13%) serous borderline, 93 (14%) mucinous borderline, 1,001 (15%) of all invasive, 567 (15%) serous invasive, 63 (17%) mucinous invasive, 155 (15%) endometrioid invasive, 93 (15%) clear cell invasive. In model 2, the reference group includes all women who did not use genital powders (nonusers and non-genital users combined).

[b]ORs were estimated using conditional logistic regression conditioned on 5-year age groups and adjusted for age (continuous), oral contraceptive duration (never use, <2, 2–<5, 5–<10, or 10 years), parity (0, 1, 2, 3, or 4+ children), tubal ligation history (no or yes), BMI (quartiles), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other).

Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

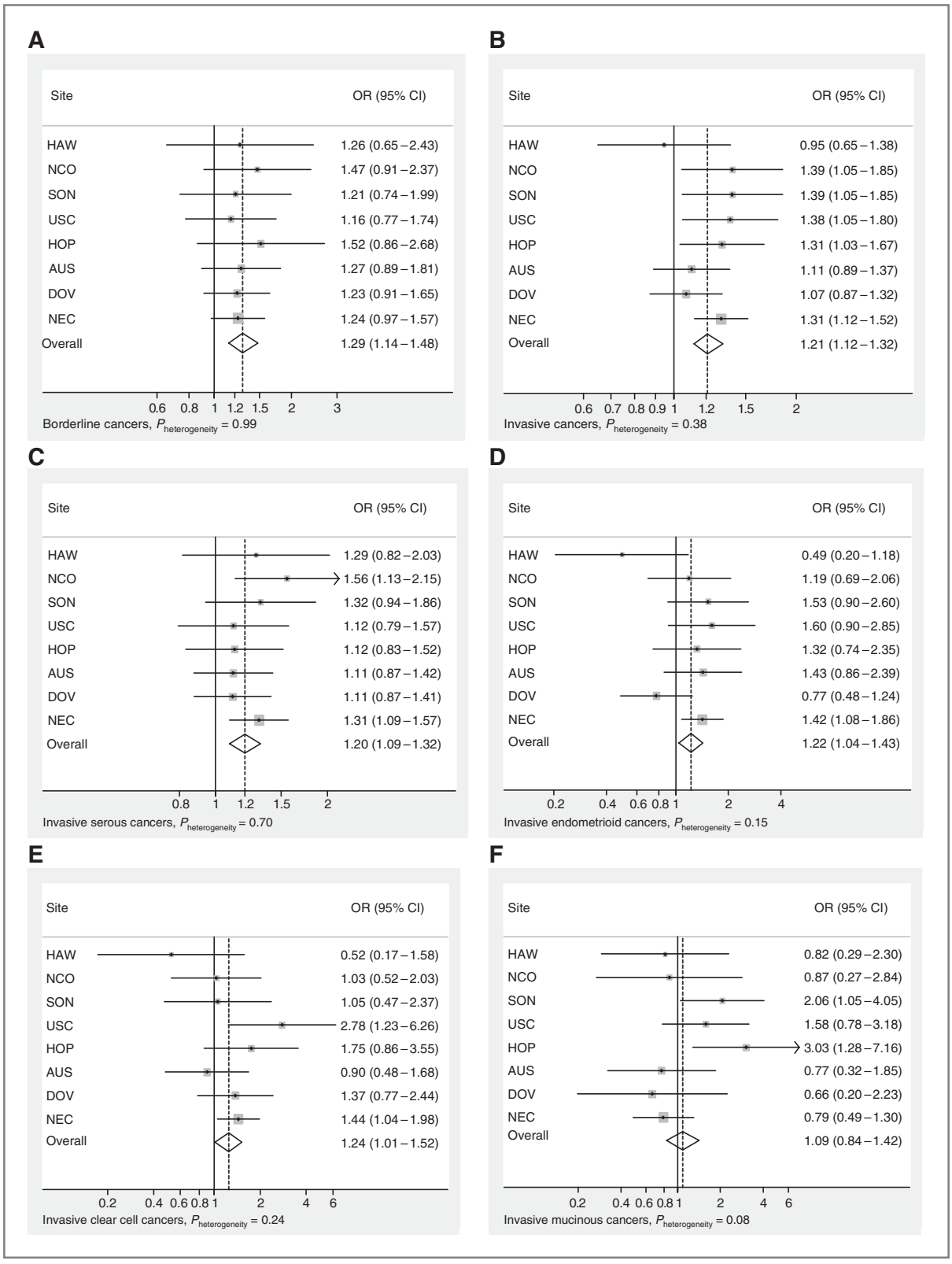

Figure 2. Association between genital powder use and subgroups of ovarian cancer defined by behavior and histology (A. Borderline, B. Invasive, C. Invasive serous, D. Invasive endometrioid, E. Invasive clear cell, F. mucinous.). Estimates are adjusted for the same covariates as in the model presented in Fig. 1.

Published OnlineFirst June 12, 2013; DOI: 10.1158/1940-6207.CAPR-13-0037

Table 5. Association between estimated lifetime applications of genital powder and risk of ovarian cancer (borderline and invasive combined)

Lifetime number of applications[a]		All cases ($N = 7,587$)		Nonmucinous cases ($N = 6,361$)	
	Controls (%)	Cases (%)	OR[b] (95% CI)	Cases (%)	OR[b] (95% CI)
Never users	6,175 (76)	5,384 (71)	1.00	4,472 (70)	1.00
Quartile 1	509 (6)	534 (7)	1.14 (1.00–1.31)	467 (7)	1.18 (1.02–1.36)
Quartile 2	512 (6)	541 (7)	1.23 (1.08–1.41)	456 (7)	1.22 (1.06–1.41)
Quartile 3	497 (6)	542 (7)	1.22 (1.07–1.40)	457 (7)	1.22 (1.06–1.40)
Quartile 4	486 (6)	586 (8)	1.32 (1.16–1.52)	509 (8)	1.37 (1.19–1.58)
P_{trend}[c]			0.17		0.17

[a]Age-specific 25th, 50th, and 75th percentile cutoff points are 612, 1,872, and 5,400 for participants < 40 years old; 612, 2,160, and 7,200 for 41–50 years; 720, 3,600, and 10,800 for 51–60 years; 1,440, 5,760, and 14,440 for 61–70; 840, 7,200, and 18,000 for > 70 years.
[b]ORs were estimated using conditional logistic regression conditioned on 5-year age groups and adjusted for age (continuous), oral contraceptive duration (never use, <2, 2–<5, 5–<10, or 10 years), parity (0, 1, 2, 3, or 4+ children), tubal ligation history (no or yes), BMI (quartiles), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other).
[c]Trend excludes never users.

we excluded the 65 cases and 79 controls who started genital powder use for the first time after surgery (OR_{Q1}, 1.19; 95% CI, 1.03 1.38; OR_{Q2}, 1.19; 95% CI, 1.03 1.38; OR_{Q3}, 1.21; 95% CI, 1.04 1.39; OR_{Q4}, 1.36; 95% CI, 1.18 1.57). For studies that collected data on timing of powder use and tubal ligation/hysterectomy, we were able to identify timing of genital powder exposure in relation to surgery based on age of powder use and age at surgery. Restricting our exposure to genital powder applications that occurred before tubal ligation or hysterectomy made no substantive difference in the results.

The association between any genital powder use and ovarian cancer risk was stronger among women with BMI < 30 kg/m^2 (OR, 1.28; 95% CI, 1.17 1.39) than for women with BMI 30 (OR, 1.14; 95% CI, 0.98 1.32; $P_{interaction} = 0.01$). We observed no significant interactions between genital powder use and parity, reported history of endometriosis, tubal ligation/hysterectomy, or menopausal status (all $P_{interaction} > 0.1$). The association between genital powder use and ovarian cancer risk was similar for women who started use between 1952 and 1961 (OR, 1.36; 95% CI, 1.19 1.56), between 1962 and 1972 (OR, 1.27; 95% CI, 1.11 1.46), and after 1972 (OR, 1.31; 95% CI, 1.15 1.51). However, we observed an attenuated association for women who started genital powder use before 1952 (OR, 1.08; 95% CI, 0.93 1.25).

Discussion

This pooled analysis of eight case control studies suggests that genital powder use is associated with a modest 20% to 30% increase in risk of developing epithelial ovarian cancer, including serous, endometrioid, and clear cell tumors, but is less relevant to invasive mucinous tumors. Our findings are consistent with and extend the findings of three meta-analyses that have reported an increased risk of epithelial ovarian cancer with genital powder use (1, 4, 5) by including dose-response and histology-specific analyses. Our estimate of the overall association between genital powder use and ovarian cancer risk was slightly attenuated compared with previous estimates from meta-analyses. Possible reasons for the difference include the lack of restriction to published results, data harmonization between studies that allowed similar definitions for the exposure and covariates, and chance. On the basis of the consistency in the epidemiologic literature on talc-based powder and ovarian cancer risk, the International Agency for Research on Cancer (IARC) classified talc-based body powder as a class 2b carcinogen "possibly carcinogenic to human beings" (29).

The biologic plausibility for the observed association between genital powder use and ovarian cancer risk has been challenged because evidence for dose-response has been inconsistent (2, 4, 5, 9, 10, 15, 22). The lack of significant dose-response may reflect the difficulty inherent in accurate recollection of specific details of frequency and duration of genital powder use. Also, because not all powder products contain talc, various products may differ in their potential carcinogenic effects. Alternatively, the association between genital powder exposure and ovarian cancer risk may not be linear and a modest exposure may be sufficient to increase cancer risk. Talc-containing powders are hypothesized to promote cancer development by ascending the female genital tract and interacting directly with the ovarian surface epithelium, leading to local inflammation characterized by increased rates of cell division, DNA repair, oxidative stress, and elevated inflammatory cytokines (13). Particles in solution easily ascend the genital tract (30, 31). Our finding of slightly attenuated associations following exclusion of women with powder exposure after tubal ligation or hysterectomy are not supportive of this hypothesis, but risk estimates in this subgroup analysis may have randomly differed from those including all women because of the reduction in

sample size. Talc particles have been observed in the ovaries of humans (32) and in rodent models (33, 34), but little is known about the biologic effects of genital powder use.

In the current analyses of the various histologic subtypes of ovarian cancer, we confirmed previous reports of increased risk of serous invasive tumors with genital powder use (2, 4, 8, 9, 22). We also observed significantly increased risk of both endometrioid and clear cell invasive ovarian tumors with use of genital powder, and this finding was consistent across studies. It has been suggested that both endometrioid and clear cell ovarian tumors may originate from ectopic uterine endometrium (endometriosis) implanted on the ovary (17). In contrast, we observed no significant associations between genital powder use and either borderline or invasive mucinous ovarian cancer. The lack of a significant association for mucinous tumors may be due to the relatively small number of these tumors or could be an indication that powder exposure is not relevant to the pathogenesis of this histologic type. Studies have noted that ovarian cancer risk factors and molecular characteristics differ for mucinous tumors (16 18, 23, 35 39).

Limitations of our pooled analysis include differences in the wording of questions about genital powder use between studies and the retrospective nature of the exposure ascertainment. Women who were classified as genital powder users varied from "ever" use (AUS) or "ever regular" use (SON) to powder use for at least 6 months (HAW, HOP, NCO, NEC, and USC) or at least 1 year (DOV). Differences in genital powder questions result in varying levels of misclassification of true genital powder exposure. However, because exposure definitions are the same for cases and controls within each study, misclassification of genital powder exposure due to the question wording would be nondifferential, leading to an underestimation of the true association for any given study. These studies were retrospective in nature and therefore potentially susceptible to bias if cases were more likely to report genital powder use than controls. Although nongenital powder use was not associated with ovarian cancer risk, it is nevertheless possible that any over-reporting of powder use by cases might have been limited to reporting of genital powder. Our analyses were also limited by missing data on genital powder use; however, missingness was not associated with the distribution of any of the ovarian cancer risk factors examined and was thus not likely to bias our results. Strengths of our analysis include a large sample size and pooled analysis of individual data, allowing evaluation of the association of genital powder use with less common histologic subgroups of ovarian cancer, careful harmonization of the data based on comparison of study questionnaires, the use of a composite variable combining duration, and frequency to assess dose response relationships.

In conclusion, our large pooled analysis of case control studies shows a small-to-moderate (20% 30%) increased risk of ovarian cancer with genital powder use, most clearly pertaining to nonmucinous epithelial ovarian tumors. More work is needed to understand how genital powders may exert a carcinogenic effect, and which constituents (e.g., talc) may be involved. Because there are few modifiable risk factors for ovarian cancer, avoidance of genital powders may be a possible strategy to reduce ovarian cancer incidence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the article.

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Acknowledgments

The authors thank all the individuals who participated in these studies as well as the researchers, clinicians, and support staff who contributed to this work. The Australian Ovarian Cancer Study Management Group (D. Bowtell, G. Chenevix-Trench, A. deFazio, D. Gertig, A. Green, and P. Webb) and AUC Investigators (A. Green, P. Parsons, N. Hayward, P. Webb, and D. Whiteman) thank all the clinical and scientific collaborators (see http://www.aocstudy.org/) and the women for their contribution.

Grant Support

Support for the OCAC was provided by donations from family and friends of the Kathryn Sladek Smith to the Ovarian Cancer Research Fund. In addition, these studies were supported by NIH (R01-CA54419, R01-CA112523, R01-CA87538, R01-CA95023, RO1-CA76016, R01-CA58598, R01-CA17054, R01-CA14089, R01-CA61132, R03-CA113148, R03-CA115195, N01-CN25403, N01-PC-67010, N01-CN55424, N01-PC67001, P50-CA105009, and P01-CA17054), the U.S. Department of Defense (DAMD17-01-1-0729, DAMD17-02-1-0669, and DAMD17-02-1-0666), National Health & Medical Research Council of Australia (199600), Cancer Council of Tasmania, Cancer Foundation of Western Australia; California Cancer Research Program (00-01389V-20170, 2II0200), and National Health Research and Development Program, Health and Welfare Canada 6613-1415-53. Individual investigators are supported by NIH [K07-CA143047 (to W. Sieh), R25-CA098566 (to M.A. Merritt)], Department of Defense [W81XWH-10-1-02802 (to K.L. Terry)], and the National Health and Medical Research Council of Australia (to P.M. Webb and C.M. Nagle).

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Received February 5, 2013; revised April 23, 2013; accepted May 21, 2013; published OnlineFirst June 12, 2013.

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Cancer Prevention Research

Genital Powder Use and Risk of Ovarian Cancer: A Pooled Analysis of 8,525 Cases and 9,859 Controls

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Cancer Prev Res 2013;6:811-821. Published OnlineFirst June 12, 2013.

Exhibit D

Perineal Talc Use and Ovarian Cancer

A Systematic Review and Meta-Analysis

Ross Penninkilampi, and Guy D. Eslick

Background: It has been posited that there is an association between perineal talc use and the incidence of ovarian cancer. To date, this has only been explored in observational studies.

Objectives: To perform a meta-analysis to evaluate the association between perineal talc use and risk of ovarian cancer.

Methods: Studies were identified using six electronic databases. Observational studies involving at least 50 cases of ovarian cancer were eligible for inclusion. We analyzed the association between ovarian cancer, including specific types, and any perineal talc use, long-term (>10 years) use, total lifetime applications, and use on diaphragms or sanitary napkins. A subgroup analysis was performed, stratifying by study design and population.

Results: We identified 24 case–control (13,421 cases) and three cohort studies (890 cases, 181,860 person-years). Any perineal talc use was associated with increased risk of ovarian cancer (OR = 1.31; 95% CI = 1.24, 1.39). More than 3600 lifetime applications (OR = 1.42; 95% CI = 1.25, 1.61) were slightly more associated with ovarian cancer than <3600 (OR = 1.32; 95% CI = 1.15, 1.50). An association with ever use of talc was found in case–control studies (OR = 1.35; 95% CI = 1.27, 1.43), but not cohort studies (OR = 1.06; 95% CI = 0.90, 1.25). However, cohort studies found an association between talc use and invasive serous type ovarian cancer (OR = 1.25; 95% CI = 1.01, 1.55). We found an increased risk of serous and endometrioid, but not mucinous or clear cell subtypes.

Conclusions: In general, there is a consistent association between perineal talc use and ovarian cancer. Some variation in the magnitude of the effect was found when considering study design and ovarian cancer subtype.

(*Epidemiology* 2018;29: 41–49)

Submitted July 12, 2017; accepted August 27, 2017.

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This manuscript is original, has not been previously published in whole or in part, and is not under consideration for publication elsewhere. Neither animals nor human subjects were used in this research.

All authors have read the manuscript, agree that the work is ready for submission, and accept the contents of the manuscript.

The authors report no conflicts of interest.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

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ISSN: 1044-3983/18/2901-0041
DOI: 10.1097/EDE.0000000000000745

Ovarian cancer is the gynecologic cancer associated with the highest mortality in the United States, in 2012 being the fifth highest cause of cancer death in women with 14,404 deaths in that country.[1] The National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER) predicts that in the United States, in 2016, there will be 22,280 incidences of newly diagnosed ovarian cancer, and 14,240 deaths caused by ovarian cancer based on age-adjusted data from 2009 to 2013.[2] The 5-year survival statistics for ovarian cancer are poor, largely because patients usually present with advanced disease, which is less amenable to curative therapy.[3] SEER estimates that only 15% of patients present with disease localized to the ovary, which contributes to a 5-year survival of 46.2%.[2] It is imperative to develop public health programs, which either reduce the incidence of ovarian cancer or detect it at an earlier stage, to reduce the burden of this disease.

Routine pelvic examinations, transvaginal ultrasonography, and tumor markers have been trialed as potential screening tools for ovarian cancer, but are limited in their usefulness. The cancer marker cancer antigen 125 (CA-125, also known as mucin 16) has been found to be elevated in 80% of all ovarian carcinomas, but this falls to 50% in women in which the cancer is localized only to the ovary, where it is most amenable to treatment.[4] As CA-125 has a low sensitivity and limited specificity, it is not recommended as a screening test for women without clinical symptoms.[5] Ultrasound has a reasonable sensitivity but poor specificity and positive predictive value, particularly as it is poor at distinguishing between benign and malignant masses.[6] While the search for an effective screening regimen for ovarian cancer continues, the importance of primary prevention becomes paramount.

Talcum powder is made of talc, a hydrated magnesium silicate, and is used to absorb moisture on the body. Some women choose to dust talc on the perineum, or apply it to diaphragms or sanitary napkins, to reduce friction, keep the skin dry, reduce odor, and prevent rashes. The potential association between perineal talc use and ovarian cancer has been discussed for decades. The first investigation of this association was performed by Cramer et al[7] in 1982, when the investigators found a relative risk of 1.92 (95% CI = 1.27, 2.89) for ovarian cancer when women either dusted the perineum with talc powder or used it on sanitary napkins. Since this time, there has been substantial interest in and research into this association.

In the present context, the association between talc use and ovarian cancer takes on considerable relevance, as the pharmaceutical and consumer products company Johnson & Johnson has recently had damages levied to the total of US$717 million against them in five law suits. In these cases, juries decided that the use of talcum powder caused or contributed to the development of the plaintiff's ovarian cancer. The evidence for the association between perineal talc use and ovarian cancer is based on the body of knowledge from observational studies, and most of these have been retrospective case–control studies prone to recall bias. Hence, while perineal talc use has not been shown to be safe, in a similar regard, a certain causal link between talc use and ovarian cancer has not yet been established.[8,9]

In 2013, a pooled analysis was performed for eight population-based case–control studies, and found a modest increased risk (OR = 1.24) of ovarian carcinoma associated with perineal talc use.[10] In 2007, a meta-analysis was performed of nine observational studies; however, this study only examined the use of talc on contraceptive diaphragms.[11] The overall finding of this meta-analysis was that the use of talc on contraceptive diaphragms was not associated with ovarian cancer. Meta-analyses have been performed on this subject before; however, the most recent was in 2008,[9] and since this time, the results of a number of large case–control studies and two cohort studies[12,13] have been published. Hence, there is a need to update the literature, particularly considering pending litigation against Johnson & Johnson by other claimants, and Johnson & Johnson's potential plans to appeal the previous decisions. Furthermore, producers of talcum powder products continue to sell these products without any warning labels regarding perineal use and potential associations with ovarian cancer. Hence, there is a need for clarification, to allow women to be adequately informed of the risk of use of these products, possibly preventing future harm.

This paper aims to review the literature and provide an overall risk estimate for the association between perineal talc use and ovarian carcinoma. We will also perform subgroup analyses by the method of talc application, the duration of talc use, the total number of perineal talc applications, and the type of ovarian cancer developed to further elucidate the relationship between talc use and ovarian carcinoma.

METHODS

Study Protocol

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[14] R.P. performed a systematic search of the databases MEDLINE (from 1950), PubMed (from 1946), Embase (from 1949), the Cumulative Index to Nursing and Allied Health Literature (CINAHL), LILACS, and the Cochrane Central Register of Controlled Trials through 22 August 2017 to identify relevant articles. The search used the terms ("talc" OR "talcum powder") AND ("ovarian cancer" OR "ovarian carcinoma"), which were searched as text word and as exploded medical subject headings where possible. We also searched the reference lists of relevant articles for appropriate studies. No language restrictions were used in either the search or study selection. We did not search for unpublished literature.

Study Selection

We included studies that met the following inclusion criteria: (1) the study investigated the perineal use of talc in relation to risk of development of ovarian cancer; (2) the study reported adverse events as an odds ratio (OR), or the data were presented such that an OR could be calculated; (3) the 95% confidence interval (CI) was reported, or the data were presented such that the CI could be calculated; and (4) the study involved a minimum of 50 cases. We excluded studies that did not meet the inclusion criteria.

Data Extraction

One of us (R.P.) performed data extraction using a standardized data extraction form, collecting information on the publication year, study design, number of cases, number of controls, total sample size, population type, country, mean age, number of adjusted variables, the risk estimates or data used to calculate the risk estimates, CIs or data used to calculate CIs, and the type of ovarian cancer. R.P. assessed the quality of the studies using the Newcastle-Ottawa Scale (NOS); however, no studies were excluded on the basis of NOS score.[15] Authors were not contacted for missing data. Adjusted ratios were extracted in preference to nonadjusted ratios; however, where ratios were not provided, R.P. calculated unadjusted ORs and CIs.

Statistical Analysis

One of us (G.D.E.) calculated pooled ORs and 95% CIs for the effect of any perineal talc use with all ovarian cancers using a random effects model.[16] Analyses were also performed based on the method of administration (diaphragm, sanitary napkins), duration of use, and type of ovarian cancer developed (all mucinous, mucinous invasive, mucinous borderline, all serous, serous invasive, serous borderline, endometrioid, clear cell). For long-term talc use, we extracted the odds ratio for the group with the longest duration of talc exposure compared with controls, provided that group used talc for a minimum duration of 10 years. For overall lifetime talc applications, groups within each study were divided into either <3600 lifetime applications, equivalent to less than approximately 10 years of daily use, or >3600 applications. Where a group from a study did not completely fit into this dichotomy, we placed it into the category it most closely fit. Details on the categorization of individual groups are available in eTable 1 (http://links.lww.com/EDE/B261). Odds ratios were pooled for invasive serous, invasive mucinous, borderline serous, and borderline mucinous tumors individually. However, as many studies reported only all mucinous or all serous in a single

group, we also ran analyses for risk associated with all mucinous and all serous tumors. Where a study reported separately as borderline and serous, both odds ratios were included separately in the meta-analysis, to ensure all available data were considered.

We tested heterogeneity with Cochran's Q statistic, with $P < 0.10$ indicating heterogeneity, and quantifled the degree of heterogeneity using the I^2 statistic, which represents the percentage of the total variability across studies which is due to heterogeneity. I^2 values of 25%, 50%, and 75% corresponded to low, moderate, and high degrees of heterogeneity, respectively.[17] We quantifled publication bias using the Egger's regression model,[18] with the effect of bias assessed using the fail-safe number method. The fail-safe number was the number of studies that we would need to have missed for our observed result to be nullifled to statistical nonsignifIcance at the $P < 0.05$ level. Publication bias is generally regarded as a concern if the fail-safe number is less than 5n + 10, with n being the number of studies included in the meta-analysis.[19] All analyses were performed with Comprehensive Meta-analysis (version 3·0; Biostat, Englewood, NJ; 2014).

RESULTS

Study Characteristics

We performed a broad literature search of electronic databases, identifying 363 citations for review (Figure 1). Initially, 318 studies were discarded, with many being narrative reviews, duplicates, animal studies, opinion pieces, editorials, or otherwise irrelevant. Forty-flve citations were selected for full-text review. Of these, three were excluded due to being associated with endometrial rather than ovarian cancer, two were meta-analyses, flve were duplications of data from the same study, one involved non-perineal application of talc, and seven were otherwise irrelevant. No studies were excluded for failing to report an odds ratio or for not providing the necessary raw data from which an odds ratio could be provided. Some studies provided only the raw data, i.e., the number of cases and controls with and without perineal talc use. This allowed an unadjusted odds ratio to be calculated, which was then included in the analysis. Overall, 27 studies were selected. Note that Wu et al[33] (2015) include results from Wu et al[36] (2009); however, only Wu et al[36] (2009) reported on non-perineal talc use, total lifetime applications, and long-term talc use. Hence data were extracted from Wu et al[33] (2015) for the "any perineal use" outcome, and from Wu et al[36] (2009) for the three other outcomes previously mentioned. Hence, while 27 studies were included in the analysis, only 26 were included in the any perineal use analysis. Three studies were cohort studies, including 890 cases and 181,860 person-years.[12,13,20] The remaining 26 studies were case–control studies, with a total of 13,421 cases and 19,314 controls. The case–control studies are described in eTable 1 (http://links.lww.com/EDE/B261), while the cohort studies are described in eTable 2 (http://links.lww.com/EDE/B261). In total, studies involving 14,311 cases of ovarian cancer were included in this review.

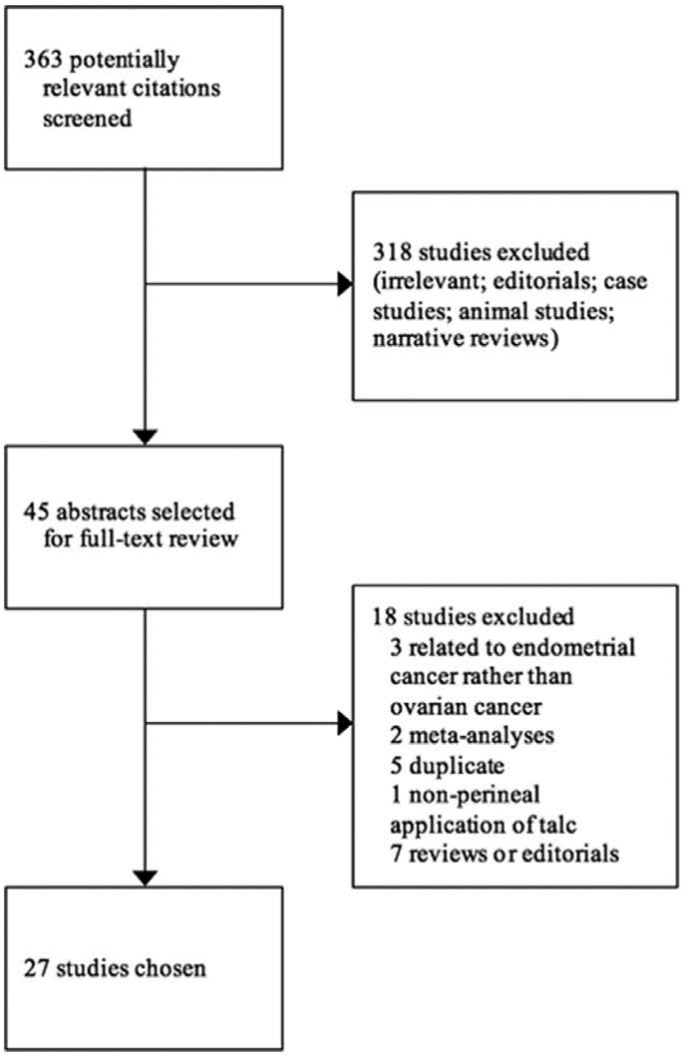

FIGURE 1. PRISMA flowchart for literature search and study selection.

The quality of the studies was assessed using the Newcastle-Ottawa Scale (NOS), which involves separate assessment tools for both case–control and cohort studies.[15] The highest score awarded was 8/10, and the lowest was 5/10. The mean score was 7.0. Almost all studies lost points because the exposure to talc was ascertained through self-report rather than an independently verifled source, and because the interviewer was not blinded to cases and controls. Many studies also failed to specifIcally describe that their chosen controls did not have a personal history of previous ovarian cancer. It may be the case that this was done, but not reported in the study methods. Generally, case ascertainment and matching controls based on age and other factors, often geographical location or ethnicity, were well performed in the reviewed studies. The breakdown of individual study scores is included in Tables 1 and 2. Overall, the quality of studies included in

TABLE 1. Summary of Pooled Effect Sizes for Examined Outcome Variables

	No. Studies	Effect Size	Heterogeneity		Publication Bias
		OR (95% CI)	I^2	*P*	*P*
Method of talc use					
Any perineal	26	1.31 (1.24, 1.39)	10.52	0.31	0.09
Any non-perineal	5	1.24 (1.01, 1.51)	66.84	0.02	0.86
Diaphragm	8	0.84 (0.68, 1.05)	14.76	0.31	0.64
Sanitary napkins	12	1.15 (0.94, 1.41)	43.82	0.05	0.17
Length of talc use					
Long-term use (>10 years)	12	1.25 (1.10, 1.43)	45.11	0.04	0.31
<3600 total applications	5	1.32 (1.15, 1.50)	1.83	0.41	0.20
>3600 total applications	5	1.42 (1.25, 1.61)	12.59	0.33	0.40
Type of ovarian cancer					
All serous	10	1.32 (1.22, 1.43)	0.00	0.75	0.44
Serous invasive	5	1.32 (1.13, 1.54)	25.10	0.25	0.75
Serous borderline	3	1.39 (1.09, 1.78)	0.00	0.94	0.83
All mucinous	9	1.12 (0.94, 1.33)	5.79	0.39	0.79
Mucinous invasive	2	1.34 (0.48, 3.79)	69.39	0.07	NA[a]
Mucinous borderline	3	1.18 (0.76, 1.81)	34.07	0.22	0.96
Endometrioid	8	1.35 (1.14, 1.60)	0.00	0.61	0.78
Clear cell	3	1.02 (0.75, 1.39)	0.00	0.78	0.22

[a]NA = not applicable; no publication bias ... result available when there are fewer than three studies in the analysis.

this review was reasonably high. No studies were excluded from the review based on NOS score.

All studies reported at least an odds ratio for any perineal use of talc and its association with ovarian cancer. As previously described, Wu et al[36] (2009) was not included in this analysis to prevent duplication of data. Five studies reported on only non-perineal exposure. Additionally, eight studies provided data for use of talc on a diaphragm, and 12 for sanitary napkins. Twelve studies provided an odds ratio for long-term talc use and its association with ovarian cancer; however, the chosen threshold for long term was variable, from more than 10 years to more than 37.4 years. Five studies reported on the total number of talc applications. It was frequently necessary to report different groups from a single study separately to perform the meta-analysis of this outcome, with the groupings being described specifically in eTable 1 (http://links.lww.com/EDE/B261). Ten studies reported odds ratios for all serous ovarian cancers, five reported for serous invasive cancers, and three reported for serous borderline cancers. Similarly, nine reported for all mucinous cancers, two for mucinous invasive, and three for mucinous borderline. Eight studies reported odds ratios for endometrioid ovarian cancer, and three reported for clear cell ovarian cancer.

Quantitative Data Synthesis

The results of the initial pooling of data from all studies are summarized in Table 1. Pooling of data revealed an increased risk of ovarian cancer associated with any perineal use of talc (Figure 2A; OR = 1.31; 95% CI = 1.24, 1.39). Use of talc long term (>10 years) was also associated with an increased ovarian cancer risk (Figure 2B; OR = 1.25; 95% CI = 1.10, 1.43). Both <3600 total lifetime applications (OR = 1.32; 95% CI = 1.15, 1.50) and >3600 lifetime applications (OR = 1.42; 95% CI = 1.25, 1.61) of talc were associated with an increased risk of ovarian cancer, with a slightly higher risk in the group with greater usage. Talc use on diaphragms or on sanitary napkins was not individually associated with increased risk of ovarian cancer. Any perineal talc use was associated with any serous (Figure 2C; OR = 1.32; 95% CI = 1.22, 1.43), serous invasive (OR = 1.32; 95% CI = 1.13, 1.54), serous borderline (OR = 1.39; 95% CI = 1.09, 1.78), and endometrioid (Figure 2D; OR = 1.35; 95% CI = 1.14, 1.60) subtypes of ovarian cancer, but not the other subtypes.

We performed a subgroup analysis stratifying by study design. It is important to note that there were only three cohort studies, each of which did not report on all the assessed associations. For any perineal talc use, only case–control studies showed an association with ovarian cancer (Figure 2A; OR = 1.35; 95% CI = 1.27, 1.43), while no association was noted for cohort studies (OR = 1.06; 95% CI = 0.90, 1.25). For the other associations assessed, the results are reported in Table 2. In cohort studies, the only association found was between perineal talc use and the incidence of serous invasive cancer subtypes (OR = 1.25; 95% CI = 1.01, 1.55). For borderline serous, borderline mucinous, invasive mucinous, and clear cell ovarian cancer subtypes, no cohort studies provided data for the association and hence the odds ratios reported in eTable 2 (http://links.lww.com/EDE/B261) are derived entirely from case–control studies. The only outcome reported in all three cohort studies was any perineal talc use; hence the available data from prospective studies were limited.

A subgroup analysis related to study population setting, i.e., in the hospital or in the general population, was performed for any perineal talc application. Generally, hospital-based studies were older (pre-2000) than the community-based studies. There were seven hospital-based studies, all of which were case–control studies. There were 20 population-based studies, including 17 case–control studies and all three cohort studies. There was no difference between the pooled results for hospital- and population-based studies (OR = 1.22 vs. 1.33), respectively.

There was heterogeneity in the analysis of non-perineal applications of talc (I^2 = 66.84; P = 0.02). There was no heterogeneity for any of the other outcome measures in either the meta-analysis of all available studies or the subgroup analyses. There was no publication bias in the meta-analysis of any genital talc exposure and ovarian cancer, which included all the studies in the review, except Wu et al[36] (2009) (Figure 3; P = 0.09). The result for publication bias for each of the individual analyses is included in Table 1.

TABLE 2. Summary of Pooled Effect Sizes in Subgroup Analysis by Study Design

	Case–Control Studies (n = 24)				Cohort Studies (n = 3)			
		Effect Size	Heterogeneity			Effect Size	Heterogeneity	
	No. Studies	OR (95% CI)	I^2	*P*	No. Studies	OR (95% CI)	I^2	*P*
Method of talc use								
Any perineal use	23	1.35 (1.27, 1.43)	0.00	0.77	3	1.06 (0.90, 1.25)	18.89	0.29
Non-perineal use	5	1.24 (1.01, 1.51)	66.84	0.02	0	NA	NA	NA
Diaphragm	7	0.81 (0.61, 1.08)	21.92	0.26	1	0.92 (0.68, 1.24)	0.00	1.00
Sanitary napkin	10	1.27 (0.98, 1.65)	40.49	0.09	2	0.93 (0.77, 1.13)	0.00	0.77
Length of talc use								
Long-term use	11	1.29 (1.13, 1.47)	40.53	0.08	1	0.98 (0.75, 1.29)	0.00	1.00
<3600 total applications	5	1.32 (1.15, 1.50)	1.83	0.41	0	NA	NA	NA
>3600 total applications	5	1.42 (1.25, 1.61)	12.59	0.33	0	NA	NA	NA
Type of ovarian cancer								
All serous	12	1.34 (1.23, 1.47)	0.00	0.71	2	1.19 (0.97, 1.47)	0.00	0.61
Serous invasive	3	1.36 (1.05, 1.75)	47.96	0.15	2	1.25 (1.01, 1.55)	0.00	0.33
Serous borderline	3	1.39 (1.09, 1.78)	0.00	0.94	0	NA	NA	NA
All mucinous	9	1.15 (0.93, 1.41)	21.03	0.26	2	0.96 (0.61, 1.53)	0.00	0.84
Mucinous invasive	2	1.34 (0.48, 3.79)	69.39	0.07	0	NA	NA	NA
Mucinous borderline	3	1.18 (0.76, 1.81)	34.07	0.21	0	NA	NA	NA
Endometrioid	6	1.39 (1.16, 1.66)	0.00	0.52	2	1.09 (0.66, 1.80)	0.00	0.48
Clear cell	3	1.02 (0.75, 1.39)	0.00	0.78	0	NA	NA	NA

NA = not applicable; no cohort studies reported on the relevant associations.

DISCUSSION

The present meta-analysis reports a positive association between perineal talc use and ovarian cancer, specifically of the serous and endometrioid histologic subtypes. The mechanism by which perineal talc use may increase the risk of ovarian cancer is uncertain. It has been previously proposed that talc, as a foreign body, may ascend from the vagina through to the uterine tubes and instigate a chronic inflammatory response, which may predispose to the development of ovarian cancer. It is argued that cellular injury, oxidative stress, and local increase in inflammatory mediators such as cytokines and prostaglandins may be mutagenic and hence promote carcinogenesis.[21] In support of this hypothesis, it has been found that hysterectomy or bilateral tubal ligation, in which ovarian exposure to inflammatory mediators would be significantly curtailed, is associated with a reduced risk of ovarian cancer.[22–24] However, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is not inversely associated with the incidence of ovarian cancer, as may be expected if the etiology was related to chronic inflammation.[25,26] It has also been found that human epithelial ovarian cells have an unusually low expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which would reduce their sensitivity to the action of NSAIDs.[27] The potential mechanism by which genital talc is associated with an increased risk of ovarian cancer hence remains unclear.

An important finding of this study is that talc use appears to be associated with increased risk of serous ovarian cancer, of both invasive and borderline types, and not with mucinous ovarian cancer. Additionally, endometrioid ovarian cancers but not clear cell cancers were significantly associated with perineal talc use. Intriguingly, a meta-analysis examining the effects of tubal ligation of ovarian cancer risk found a reduced risk of the same subtypes of ovarian cancer as mentioned here: serous and endometrioid, but not mucinous.[24] If chronic inflammation due to ascending foreign bodies is indeed the mechanism by which talc use is associated with increased ovarian cancer risk, then these results fit the picture. The results for non-perineal application of talc were still positive but of lower magnitude, supporting the hypothesis of ascending foreign bodies causing chronic inflammation. It is plausible that non-perineal application of talc may cause increased risk through, e.g., the respiratory tract. Unfortunately, the evidence remains insufficient to understand the mechanism with any reasonable certainty.

We also found a slightly greater increased risk of ovarian cancer with >3600 lifetime applications compared with those with <3600 lifetime applications. The number of lifetime applications is a more valid measure of the patient's exposure to perineal talc than either duration or frequency of use alone. This finding also supports the chronic inflammatory hypothesis, as repeated exposure would induce a longer period of chronic inflammation, and therefore should increase the predisposition to the development of ovarian cancer. It is notable that these data were only available from

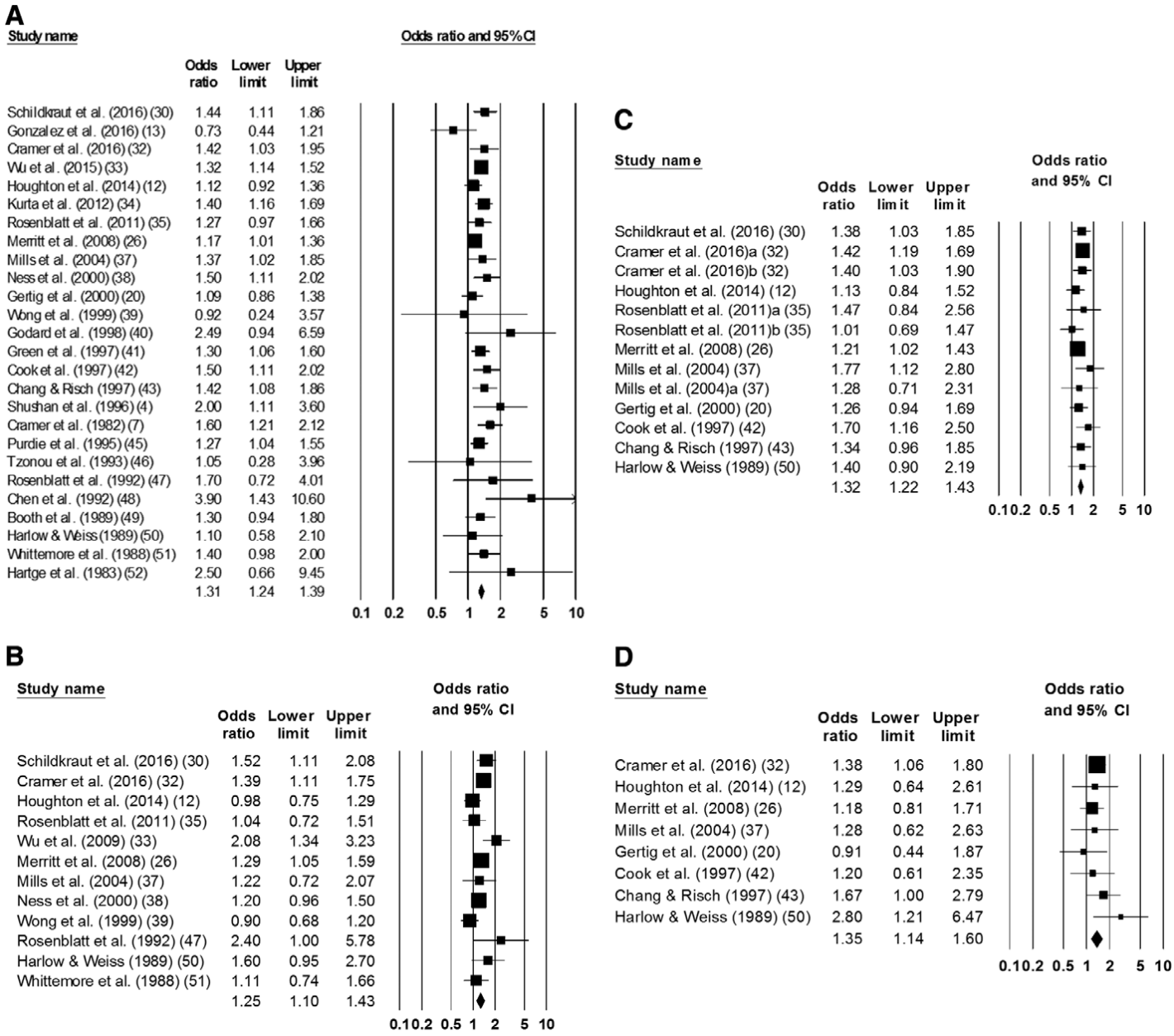

FIGURE 2. A, Any perineal talc use is associated with an increased risk of any ovarian cancer (OR = 1.31; 95% CI = 1.24, 1.39). B, Long-term perineal talc use (>10 years use) is associated with an increased risk of any ovarian cancer, but of a lower magnitude than any perineal use (OR = 1.25; 95% CI = 1.10, 1.43). C, Any perineal talc use is associated with an increased risk of serous ovarian cancers (OR = 1.32; 95% CI = 1.22, 1.43). D, Any perineal talc use is associated with an increased risk of endometrioid type ovarian cancers (OR = 1.35; 95% CI = 1.14, 1.60).

case–control studies, as the three cohort studies did not sufficiently record duration and frequency of use to be included in the analysis. This retrospective finding is therefore prone to recall bias.

This meta-analysis had several strengths. None of the analyses in this review had statistically significant heterogeneity, except for non-perineal application, which indicates consistency in the direction and magnitude of the effect size between individual studies, and strengthening the reliability of the pooled effect sizes. Another strength of this review is the large number of overall cases (n = 14,311), improving the power of the meta-analysis to detect a relatively small effect size, as occurred in this case. Another strength of this review is that the included studies were of relatively high quality as assessed through the NOS, reducing the potential for bias in the conclusions drawn. The NOS revealed that the most common limitations of the included case–control studies were the failure to blind interviewers to case–control status of subjects in the interview, and reliance on memory and self-report for collection of data on perineal talc use.

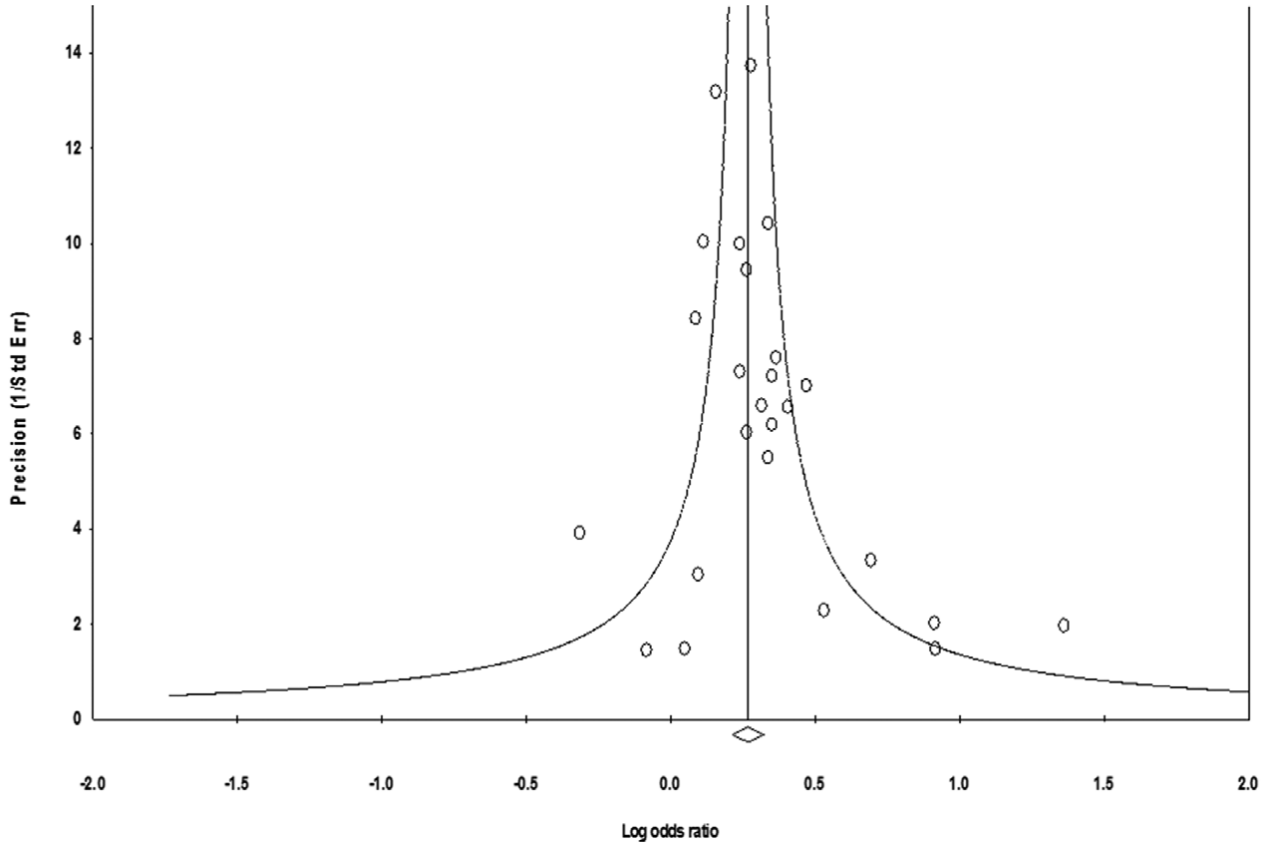

FIGURE 3. Funnel plot for the meta-analysis of studies examining any perineal talc use and risk of ovarian cancer ($P = 0.09$).

A limitation of this study is that it pools nonrandomized studies, primarily case–control studies. The retrospective nature of case–control studies introduces the potential for recall bias. In this case, it is entirely possible that patients with ovarian cancer may be more aware of their previous talc use and hence be more likely to report higher past use. It is possible to attempt to overcome this by blinding the participants to the nature of the study, usually by asking spurious questions; however, the effectiveness of this approach may be limited.[28] Many of the studies in this review recorded data about talc use as part of a more extensive questionnaire focused on other associations, which may reduce the potential for recall bias. However, since the initiation of lawsuits in 2014, there has been extensive media coverage regarding this association, and the potential for recall bias in case–control studies conducted since then may be exacerbated.

Cohort studies are useful in that they are prospective; however, the low incidence of ovarian cancer results in relatively small number of cases even in large cohorts, as seen in the three cohort studies included in this review.[29] Considering potential exposure misclassification issues in case–control studies, the effect for any perineal talc use was very weak in a small number of cohort studies. However, an association between talc use and serous invasive ovarian cancer was found.

Of the studies in this review, case–control studies achieved much large number of cases, in some instances in excess of 2000 cases and a similar number of age-matched controls, which provide greater statistical power for the detection of an effect size of small magnitude. Hence while case–control studies are low-level evidence, they have been preferred in the investigation of the association between talc use and ovarian cancer. They also have the important advantage of not requiring 15 or more years of follow-up, as is necessary for a cohort study to sufficient detect cases of ovarian cancer relative to certain exposures. One potential way to overcome this limitation in future studies is to ensure that talc use is always included in questionnaires of any cohort studies investigating ovarian cancer. It is important not only that talc use be investigated but also the precise location, duration, and frequency of use. As it stands, a meta-analysis of observational studies such as the present study provides the highest level of evidence practically feasible for this research question.

CONCLUSIONS

The results of this review indicate that perineal talc use is associated with a 24%–39% increased risk of ovarian cancer. While the results of case–control studies are prone to recall bias, especially with intense media attention following the commencement of litigation in 2014, the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association. Additional epidemiologic evidence from prospective

studies with attention to effects within ovarian cancer subtype is warranted. There is a substantial need for further research on a potential mechanism by which ovarian cancer may be caused by talc, as this will allow a causal relationship to be established or rejected with more certainty. However, particularly because of the dearth of screening tests available for this high-mortality cancer, it is important that research into this association continue as it is a potential avenue for cancer prevention.

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Exhibit E

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

Research Article

Cancer Epidemiology, Biomarkers & Prevention

Association between Body Powder Use and Ovarian Cancer: The African American Cancer Epidemiology Study (AACES)

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Abstract

Background: Epidemiologic studies indicate increased ovarian cancer risk among women who use genital powder, but this has not been thoroughly investigated in African American (AA) women, a group with a high prevalence of use. We evaluate the relationship between use of genital powder and nongenital powder in invasive epithelial ovarian cancer (EOC).

Methods: Subjects are 584 cases and 745 controls enrolled in the African American Cancer Epidemiology Study (AACES), an ongoing, population-based case–control study of EOC in AA women in 11 geographic locations in the United States. AA controls were frequency matched to cases on residence and age. Logistic regression was used to calculate ORs and 95% confidence intervals (CI) for associations between genital and nongenital powder exposure and EOC risk, controlling for potential confounders.

Results: Powder use was common (62.8% of cases and 52.9% of controls). Genital powder was associated with an increased risk of EOC (OR = 1.44; 95% CI, 1.11–1.86) and a dose–response relationship was found for duration of use and number of lifetime applications ($P < 0.05$). Nongenital use was also associated with EOC risk, particularly among nonserous EOC cases (OR = 2.28; 95% CI, 1.39–3.74). An association between powder use and upper respiratory conditions suggests an enhanced inflammatory response may explain the association between body powder and EOC.

Conclusions: In a study of AA women, body powder use was significantly associated with EOC risk.

Impact: The results support that body powder is a modifiable risk factor for EOC among AA women. *Cancer Epidemiol Biomarkers Prev; 25(10); 1411–7. ©2016 AACR.*

See related commentary by Trabert, p. 1369

Introduction

Genital powder use may be a modifiable risk factor for epithelial ovarian cancer (EOC), the most deadly of all gynecologic cancers (1). In 2010, the International Agency for Research on Cancer (IARC) classified perineal (genital) use of nonasbestos–containing, talc-based body powder as "possibly" carcinogenic to humans (2). Although particles of asbestos have been found in older body powder formulations, particularly prior to 1976 (3), more recent body powder formulations no longer contain asbestos (4, 5). However, the relationship between genital powder use and ovarian cancer appears to persist (6). It has been proposed that talc-containing powders may promote cancer development through local inflammation, increased rates of cell division and DNA repair, increased oxidative stress, and increased cytokine levels (7).

A recent pooled analysis of eight population-based case–control studies demonstrated an elevated OR of 1.24 for the association between genital powder use and EOC (6). Some (7–15) but not all (6, 8, 16) previously published studies of talc and ovarian cancer reported a dose–response relationship with genital powder use for frequency, duration, or number of applications. In addition, some studies reported a stronger association among the most common serous histologic subtype (4, 10, 14, 16, 17) although the pooled analysis did not confirm this finding (6). Only one prospective study (17) found a significant association with ever genital talc use and invasive serous EOC (RR = 1.40; 95% CI, 1.02–1.91), although no overall association with EOC was found. The Women's Health Initiative (WHI; ref. 18) did not detect an association with

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doi: 10.1158/1055-9965.EPI-15-1281

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

genital talc use and EOC. Neither prospective study found evidence of a dose–response relationship.

Previous studies of genital powder use have included mostly white women. However, two studies reported analyses stratified by race and both found an increased EOC risk among African American (AA) women who used genital talc (14, 15). One study reported a nonsignificant association between one or more years of talc use and risk of ovarian cancer, OR = 1.56, [95% confidence interval (CI), 0.80–3.04] among a small sample of 128 AA EOC cases and 143 AA controls, who were shown to have higher prevalence of talc use compared with whites (14). A second study reported an imprecise but significant association with genital talc use with an OR of 5.08 (95% CI, 1.32–19.6) among a very small sample of 16 cases and 17 controls (15). In this article, we present analyses of the relationship between both genital powder and nongenital powder exposure from the African American Cancer Epidemiology Study (AACES), an ongoing, multicenter case–control study of invasive EOC in AA women.

Materials and Methods

Study population

AACES is an ongoing, population-based, case–control study of invasive EOC in AA women in 11 locations (Alabama, Georgia, Illinois, Louisiana, Michigan, New Jersey, North Carolina, Ohio, South Carolina, Tennessee, and Texas). Institutional review board approval was obtained from all participating institutions. Methods have been described in detail elsewhere (19). Briefly, cases include AA women 20 to 79 years of age with newly diagnosed EOC. With a goal of enrolling an equal number of cases and controls, controls were AA women identified through random digit dialing, with at least one intact ovary and no history of ovarian cancer, and frequency matched to cases on region of residence and 5-year age categories. Participants complete a baseline telephone interview, which includes detailed questions on demographic characteristics; reproductive, gynecologic, and medical history; hormone therapy (HT) and oral contraceptive (OC) use; cancer family history and lifestyle characteristics including smoking, alcohol consumption, and physical activity. In an effort to obtain information from as many women as possible, a short version of the questionnaire is offered to those who would otherwise refuse to participate in the study. Accrual began in December 2010 and as of August 31, 2015, 593 cases and 750 controls were enrolled. Eligibility for this analysis was restricted to participants for whom data on body powder use and all covariates were available, resulting in a final sample size of 584 cases and 745 controls; of these, 49 cases and 16 controls completed the short questionnaire.

Exposure to body powder and talc

In the baseline interview, participants were asked whether they had ever regularly used talc, cornstarch, baby, or deodorizing powders. Participants were considered "regular users" if they reported using any of these powders at least one time per month for at least 6 months, and "never users" if they did not. Regular users were asked about their frequency and duration of use, age at first use, and whether they applied powders to genital areas (including on underwear or sanitary napkins, or on birth control devices like diaphragms) and/or nongenital areas. Participants were categorized according to their type of application as nongenital use only, genital use only, or genital and nongenital use. Lifetime number of applications was calculated by multiplying the number of body powder applications per month by the number of months used. Occupational exposure to talc (yes, no) was available only for subjects completing the long baseline survey.

Statistical analysis

The prevalence of demographic characteristics was calculated and t tests and χ^2 tests were performed to compare distributions between cases and controls. Because of the relatively small number of women who reported having only used genital powder (43 cases and 44 controls), we merged this exposure category with those who reported use of both nongenital and genital powder, creating an exposure category of "any" genital powder use. Unconditional multivariable logistic regression was performed to calculate ORs and 95% CIs for the associations between body powder exposure ("only" nongenital use, and "any" genital use) and risk of EOC. Body powder exposure was further examined by frequency of use (less than 30 times per month, daily), duration of use categorized as less than the median or the median and greater among the controls (<20 years, 20 years), and lifetime number of applications categorized as less than the median or the median and greater among controls (<3,600, 3,600 lifetime applications). Trend tests for frequency, duration, and lifetime applications of powder use by route of exposure were conducted separately in two subsamples: only nongenital users plus never users and any genital users plus never users. For each subsample, each of the above variables was entered into a logistic regression as multiple indicator variables representing three levels and two degrees of freedom (i.e., for frequency of use: no exposure, less than daily, daily), adjusting for confounders. Trends were evaluated by statistical tests for the association between frequency/duration/lifetime applications with EOC risk, using Wald tests to simultaneously test the equality of parameter estimates with zero. Because experimental data suggest a relationship between inhaled inert particles and asthma (20), a logistic regression analysis was conducted to determine the association between body powder use and upper respiratory conditions (yes/no), controlling for EOC case/control status.

Covariates included reference age in years (age at diagnosis for cases and age at baseline interview for controls); study site [Alabama, Louisiana, New Jersey, North Carolina, Ohio, South Carolina, Texas, Michigan and Illinois (combined because of sample size and regional similarities), Georgia and Tennessee (combined because of sample size)]; education (high school, some after high school training, college or graduate degree); parity (0, 1, 2, 3+); duration of oral contraceptives (never, <60 months, 60 months); history of tubal ligation (yes/no); family history of breast or ovarian cancer in a first-degree relative (yes/no); smoking (ever/never); and body mass index (BMI < 25, 25–29.9, 30 kg/m^2). Two class action lawsuits were filed in 2014 (21) concerning possible carcinogenic effects of body powder, which may have influenced recall of use. Therefore, year of interview 2014 or later (yes/no) was included as a covariate in the logistic regression models. To assess potential reporting bias, we also examined whether there were differences in prevalence of reported powder use by interview year (before 2014, 2014 and later) for cases and controls as well as whether interview year was an effect modifier of the relationship between powder use and EOC risk.

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

Analyses by the histologic subtype versus all controls were also conducted and heterogeneity of risk estimates was tested by seemingly unrelated regression (22). Because of the missing data for histology, 48 cases were omitted from these analyses. Through stratified analyses, we also assessed possible effect modification of the association with powder use and ever use of HT among postmenopausal women using logistic regression. Experimental data show that the inflammatory response is enhanced in the presence of estrogen and progesterone and we therefore tested for interaction of the association with body powder use by menopausal status (20). Logistic regression and trend analyses were performed using SAS version 9.4 (SAS Institute).

Results

Descriptive statistics for cases and controls are presented in Table 1. Cases were older than controls and had lower educational achievement. Although this study was designed to match controls to cases by 5-year age group, the difference in the age at diagnosis/age at interview may, in part, be because the study is actively enrolling subjects. However, age ranges of cases (20–79 years) and controls (20–79 years) overlap. Significant differences in the distributions of well-established risk factors, including a shorter duration of oral contraceptive use, and lower prevalence of tubal ligation in cases as compared with controls, were as expected. As expected, parity was lower among cases compared with controls, but the difference was not significant. In addition, cases were more likely to report a family history of breast or ovarian cancer. No significant difference in the median years of use of body powder or occupational exposure of talc in cases compared with controls was observed.

Table 2 shows the results of logistic regression models examining the relationship between any use of body powder (either "only" nongenital powder or "any" genital powder) as well as the use of body powder by type of application: "only" nongenital powder use or "any" genital powder use. Adjusting for potential confounders, we observed a significant positive association between any powder use and EOC (OR = 1.39; 95% CI, 1.10–1.76). The OR for the association with "any" genital powder use was 1.44 (95% CI, 1.11–1.86). An OR of 1.31 (95% CI, 0.95–1.79) for the measure of association between "only" nongenital powder use and EOC was only slightly lower in magnitude compared with the association when "any" genital use was reported, but not statistically different from one another ($P = 0.56$). In 2014 and later, we observed an increase in any powder use of 12% and 6% of cases and controls, respectively. Although increased, these exposure prevalences were not significantly different from those interviewed before 2014 ($P = 0.30$). For those interviewed in 2014 or later, we observed an OR for "any" genital powder use of 2.91 (95% CI, 1.70–4.97) compared with 1.19 (95% CI, 0.87–1.63) before 2014. We observed a weaker OR of 1.26 (95% CI, 0.69–2.32) for 2014 and later compared with 1.40 (95% CI, 0.96–2.03) before 2014 for those who reported "only" nongenital use. A test for effect modification by year of interview was statistically significant ($P = 0.005$).

The ORs for the association between daily use of powder for either "only" nongenital powder use (OR = 1.53; 95% CI, 1.00–2.35) or "any" genital powder use (OR = 1.71; 95% CI, 1.26–2.33) with EOC were larger in magnitude than ORs for less than daily use compared with never use but the test for trend was significant for only "any" genital powder use (Table 2). There is a moderately stronger association for 20 years of "any" genital powder use (OR = 1.51; 95% CI, 1.11–2.06) compared with <20 years of use (OR = 1.33; 95% CI, 0.95–1.86; $P_{trend} = 0.02$). No dose–response with years of use was detected for "only" nongenital powder use. The ORs for the number of lifetime applications

Table 1. Characteristics of ovarian cancer cases and controls in the African American Cancer Epidemiology Study (AACES)

	Cases ($n = 584$) n (%)	Controls ($n = 745$) n (%)	P
Age (years)			<0.01
<40	31 (5.3)	80 (10.7)	
40–59	299 (51.21)	398 (53.4)	
60+	254 (43.5)	267 (35.8)	
Range (years)	20–79	20–79	
Education			0.02
High school or less	262 (44.9)	278 (37.3)	
Some after high school training	145 (24.8)	210 (28.2)	
College or graduate degree	177 (30.3)	257 (34.5)	
Body mass index (kg/m^2)			0.09
<24.9 (under- and normal weight)	86 (14.7)	140 (18.8)	
25–29.9 (overweight)	148 (25.3)	197 (26.4)	
>30 (obese)	350 (59.9)	408 (54.8)	
Parity (# of live births)			0.06
0	105 (18.0)	96 (12.9)	
1	113 (19.4)	141 (18.9)	
2	136 (23.3)	198 (26.6)	
3+	230 (39.4)	311 (41.6)	
Tubal ligation			0.02
Yes	201 (34.4)	302 (40.5)	
No	383 (65.6)	443 (59.5)	
Oral contraceptive use			<0.01
Never	180 (30.8)	155 (20.8)	
<60 months	230 (39.4)	334 (44.8)	
>60 months	174 (29.8)	256 (34.4)	
First-degree family history of breast or ovarian cancer			<0.01
Yes	149 (25.5)	132 (17.7)	
No	435 (74.5)	613 (82.3)	
Menopausal status			0.31
Premenopausal	158 (27.2)	221 (29.7)	
Postmenopausal	423 (72.8)	522 (70.3)	
Hormone therapy			0.10
Ever use	118 (20.3)	125 (16.8)	
Never use	463 (79.7)	618 (83.2)	
Smoking			0.48
Ever	257 (44.0)	313 (42.0)	
Never	327 (56.0)	432 (58.0)	
Hysterectomy[a]			0.43
Yes	141 (24.1)	166 (22.3)	
No	443 (75.9)	579 (77.7)	
Body powder use (median years)[b]	20	20	0.48
Occupational talc exposure[c]			0.16
Yes	58 (10.8)	62 (8.5)	
No	477 (89.2)	667 (91.5)	
Histologic subtype[d]			
Serous	393 (73.2)		
Mucinous	24 (4.5)		
Endometrioid	72 (13.4)		
Clear cell	13 (2.4)		
Other	35 (6.5)		

[a]Defined as hysterectomy 2 years prior to diagnosis for cases and 2 years prior to interview for controls.
[b]Among body powder ever users only.
[c]Data not available for participants who completed the short questionnaire (49 cases and 16 controls).
[d]Data missing on histologic subtype for 47 cases.

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

Table 2. Adjusted ORs for the associations between mode, frequency, and duration of body powder use and ovarian cancer in the AACES

Exposure	Cases ($n = 584$) *n* (%)	Controls ($n = 745$) *n* (%)	OR[a] (95% CI)
Body powder use			
Never use	217 (37.2)	351 (47.1)	1.00 (Referent)
Ever use	367 (62.8)	394 (52.9)	1.39 (1.10–1.76)
Body powder use by location			
Never use	217 (37.2)	351 (47.1)	1.00 (Referent)
Only nongenital use	119 (20.4)	140 (18.8)	1.31 (0.95–1.79)
Any genital use	248 (42.5)	254 (34.1)	1.44 (1.11–1.86)
Interview date <2014	($n = 351$)	($n = 571$)	
Never use	147 (41.9)	286 (48.4)	1.00 (Referent)
Only nongenital use	76 (21.7)	104 (17.6)	1.40 (0.96–2.03)
Any genital use	128 (36.5)	201 (34.0)	1.19 (0.87–1.63)
Interview date >2014	($n = 233$)	($n = 154$)	
Never use	70 (30.0)	65 (42.2)	1.00 (Referent)
Only nongenital use	43 (18.4)	36 (23.3)	1.26 (0.69–2.32)
Any genital use	120 (51.5)	53 (34.4)	2.91 (1.70–4.97)
Frequency of use			
Never use	217 (37.3)	351 (47.2)	1.00 (Referent)
Only nongenital use			
Less than daily	61 (10.5)	82 (11.0)	1.15 (0.78–1.71)
Daily	58 (10.0)	58 (7.8)	1.53 (1.00–2.35)
P_{trend}			0.09
Any genital use			
Less than daily	88 (15.1)	119 (16.0)	1.12 (0.80–1.58)
Daily	158 (27.2)	134 (18.0)	1.71 (1.26–2.33)
P_{trend}			<0.01
Duration of use			
Never use	217 (37.4)	351 (47.4)	1.00 (Referent)
Only nongenital use			
<20 years	59 (10.2)	68 (9.2)	1.37 (0.91–2.07)
>20 years	60 (10.3)	70 (9.5)	1.28 (0.85–1.93)
P_{trend}			0.13
Any genital use			
<20 years	101 (17.4)	118 (15.9)	1.33 (0.95–1.86)
>20 years	144 (24.8)	134 (18.1)	1.52 (1.11–2.07)
P_{trend}			0.02
Lifetime body powder applications			
Never use	217 (37.4)	351 (47.4)	1.00 (Referent)
Only nongenital use			
Below median (<3,600 applications)	60 (10.3)	72 (9.7)	1.35 (0.90–2.03)
Above median (>3,600 applications)	59 (10.2)	66 (8.9)	1.30 (0.86–1.97)
P_{trend}			0.14
Any genital use			
Below median (<3,600 applications)	92 (15.9)	119 (16.1)	1.16 (0.83–1.63)
Above median (>3,600 applications)	152 (26.2)	133 (17.9)	1.67 (1.23–2.26)
P_{trend}			<0.01

[a]Adjusted for age at diagnosis/interview, study site, education, tubal ligation, parity, BMI, duration of OC use, first-degree family history of breast or ovarian cancer, and interview year.

of body powder at or above and below the median support a dose–response with "any" genital powder use ($P_{trend} < 0.01$) but not for nongenital powder use ($P_{trend} = 0.14$).

A report of any occupational talc exposure, for those completing the long baseline questionnaire, was found to be positively, but not statistically significantly, associated with EOC (OR = 1.31; 95% CI, 0.88–1.93; data not shown). Table 3 shows an OR of 1.38 (95% CI, 1.03–1.85) for the association in serous cases with "any" genital powder use. Among serous cases, the OR for "only" nongenital powder use was lower in magnitude and not significant (OR = 1.10; 95% CI, 0.76–1.58). Compared with serous cases, larger and statistically significant ORs are found for the associations with type of powder application in nonserous EOC cases; ORs were 1.63 (95% CI, 1.04–2.55) and 2.28 (95% CI, 1.39–3.74), for "any" genital powder use and "only" nongenital powder use, respectively (Table 3). A comparison of adjusted odds ratios between serous and nonserous histologic subtypes and powder use, detected a difference in "only" nongenital powder use ($P = 0.008$), but did not detect significant differences in association for "any" genital powder use ($P = 0.50$).

The stratified results by menopausal status (Table 4) suggest differences in the association for exposure to "only" nongenital powder use among premenopausal where no association is seen for "only" nongenital powder use, whereas the association with the risk of EOC and "any" genital use is elevated. Among postmenopausal women, we observed positive associations of similar magnitude for both the association between EOC and "only" nongenital powder use (OR = 1.49; 95% CI, 1.04–2.15) and "any" genital powder use (OR = 1.41; CI, 1.03–1.92). However, tests of interaction indicate no evidence for interaction by menopausal status for either route of exposure. Among menopausal women, analyses stratified by HT use suggest a stronger association among users compared with nonusers of HT for both routes of applications, although we detected a borderline, nonsignificant interaction for the associations with "any" genital body powder by HT use ($P = 0.06$). The test for interaction for nongenital body powder by HT use was not significant ($P = 0.76$).

To further consider the underlying mechanism for the relationship between use of body powder and the risk of EOC, we calculated the association between both "only" nongenital powder use and "any" genital powder use and having an upper respiratory condition. Controlling for case–control status, age at diagnosis/interview, study site, education, smoking, and BMI, we found ORs of 1.35 (95% CI, 0.89–2.05) and 1.45 (95% CI, 1.03–2.05) for "only" nongenital and "any" genital powder use, respectively, in relation to a reported respiratory condition, respectively (data not shown). A nonsignificant, but elevated OR of 1.26 (95% CI, 0.77–2.06) was observed with occupational exposure to talc and respiratory conditions (data not shown).

Table 3. Adjusted ORs for the associations between talc use and serous/nonserous EOC

Histologic subtype[a]	Cases *n* (%)	Controls *n* (%)	OR[b] (95% CI)
Serous ($n = 392$)			
Never use	156 (39.8)	351 (47.1)	1.00 (Referent)
Only nongenital use	71 (18.1)	140 (18.8)	1.10 (0.76–1.58)
Any genital use	165 (42.1)	254 (34.1)	1.38 (1.03–1.85)
Nonserous ($n = 144$)			
Never use	44 (30.6)	351 (47.1)	1.00 (Referent)
Only nongenital use	42 (29.2)	140 (18.8)	2.28 (1.39–3.74)
Any genital use	58 (40.3)	254 (34.1)	1.63 (1.04–2.55)

[a]Test for interaction for association with powder use by serous and nonserous histologic subtype and route of body powder exposure was $P = 0.008$ for "only" nongenital powder use and $P = 0.50$ for "any" genital powder use.

[b]Adjusted for age at diagnosis/interview, study site, education, tubal ligation, parity, BMI, duration of OC use, first-degree family history of breast or ovarian cancer, and interview year.

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

Table 4. Adjusted ORs for the association between EOC risk and body powder by menopausal status and HT use

	Premenopause			Postmenopause		
Exposure	Cases ($n = 158$) *n* (%)	Controls ($n = 221$) *n* (%)	OR[a] (95% CI)	Cases ($n = 423$) *n* (%)	Controls ($n = 522$) *n* (%)	OR[a] (95% CI)
Body powder use[b]						
Never use	59 (37.3)	103 (46.6)	1.00 (Referent)	157 (37.1)	247 (47.3)	1.00 (Referent)
Only nongenital use	22 (13.9)	42 (19.0)	0.90 (0.44–1.84)	97 (22.9)	98 (18.8)	1.49 (1.04–2.15)
Any genital use	77 (48.7)	76 (48.7)	1.50 (0.87–2.57)	169 (40.0)	177 (33.9)	1.41 (1.03–1.92)
HT ever/never use[c,d,e]						
HT ever use						
Never use				34 (32.1)	55 (48.7)	1.00 (Referent)
Only nongenital use				23 (21.7)	23 (20.4)	1.74 (0.77–3.92)
Any genital use				49 (46.2)	35 (31.0)	2.68 (1.33–5.40)
HT never use						
Never use				122 (38.9)	191 (46.9)	1.00 (Referent)
Only nongenital use				73 (23.3)	75 (18.4)	1.51 (0.99–2.29)
Any genital use				119 (37.9)	141 (34.6)	1.24 (0.87–1.79)

[a]Adjusted for age at diagnosis/interview, study site, education, tubal ligation, parity, BMI, duration of OC use, first-degree family history of breast or ovarian cancer, and interview year.
[b]Test for interaction between menopausal status and route of body powder exposure was nonsignificant for only non-genital use ($P = 0.21$) and any genital use ($P = 0.85$) compared with never use.
[c]Restricted to postmenopausal women.
[d]Test for interaction between HT use and only nongenital use was nonsignificant ($P = 0.76$).
[e]Test for interaction between HT use and any genital use was nonsignificant ($P = 0.06$).

Discussion

In the largest EOC case–control study in AA women to date, we observed a positive association between regular use of powder and EOC regardless of the route of application. Users of genital powder were shown to have greater than a 40% increased risk of EOC compared with an increased risk of more than 30% among those who used only nongenital powder. The OR for the association with genital powder use in the current study is consistent with the association reported in AA women by Wu and colleagues (14). Of note, a high proportion of EOC cases (63%) and controls (53%) reported any use of body powder. A dose–response trend was evident for median years of use or greater as well as median number or greater of lifetime applications of "any" genital powder but not for use of "only" nongenital powder. Our results support that the association with "any" genital powder use is similar in premenopausal and postmenopausal women, whereas there appears to be an association with use of "only" nongenital powder use among postmenopausal but not premenopausal women. Associations were found among nonserous EOC cases and among postmenopausal users of HT exposed to either genital or nongenital powder.

Most previous case–control studies have not found an association between nongenital powder use and ovarian cancer, including a large pooled analysis by Terry and colleagues who reported an adjusted OR of 0.98 (95% CI, 0.89–1.07; refs. 6, 16). No prospective studies have evaluated nongenital powder use, nor has any study examined these associations by histologic subtype (17, 18). In the current study, the overall association with nongenital use and EOC was similar to that for genital powder use though it did not reach statistical significance possibly due to small numbers and random variation. However, we also did not find a dose–response relationship with frequency, duration, or lifetime applications of "only" nongenital powder use. Furthermore, we did not detect a significant association with use of "only" nongenital powder among serous cases, whereas the OR for the association with use of "only" nongenital powder showed over a 2-fold significant increased risk for nonserous EOC. In fact, we found a statistically significant difference between associations by subtype for "only" nongenital use. Given the inconsistency with previous published findings, it is also reasonable that underreporting genital powder use, such as abdominal powder use that reaches the genital area, may have led to a spurious result. Another possible explanation for our finding may be that there is a higher inflammatory response in AAs compared with whites (23–25). Our results also suggest that the route of powder exposure may have different effects by histologic subtype. As most high-grade serous EOC, but not nonserous subtypes, arise in the fallopian tubes (26), it is possible that direct exposure through the genital tract specifically affects this disease subtype. The association with any genital powder use and nonserous cases may be due to the overlap between genital and nongenital powder use (83% of cases and 83% of controls). We were unable to examine associations with "only" genital powder users due to sample size considerations. In contrast, nongenital powder use may be related to inhalation of the exposure through the lungs. Several large pooled analyses have demonstrated risk factor associations with inflammatory-associated exposures, such as smoking (27), endometriosis (28), and obesity (29) with nonserous histologic subtypes of ovarian cancer but not high-grade serous EOC, providing a plausible theoretical basis for differences we found in associations by histologic subtype.

Akin to talc powders, titanium dioxide (TiO_2) is another inert particle that induces an inflammatory response upon inhalation and has been considered to be "possibly carcinogenic to humans" by IARC (2). Experimental evidence of enhanced inflammation due to exposure to inert environmental particulates of TiO_2 showed inhibition of phagocytic activity of alveolar macrophages in pregnancy, and was found to be associated with increased asthma risk in the offspring of BALB/c mice exposed to TiO_2. In this study, elevated estrogen levels during pregnancy were found to contribute to the resulting asthma risk (20). Our findings also support that enhanced airway inflammation is due to exposure to inert particles.

Published OnlineFirst May 12, 2016; DOI: 10.1158/1055-9965.EPI-15-1281

Consistent with a recent study (15) where an association with powder use and asthma was reported, the relationship between body powder use and respiratory conditions likely reflects an enhanced inflammatory response due to powder use, suggesting a mechanism by which EOC risk is increased. Therefore, lung inhalation of powder could be a biologically plausible mechanism for the association between nongenital body powder use and increased EOC risk, particularly in nonserous EOC cases.

To further explore whether estrogen influences the inflammatory response, we performed stratified analyses by menopausal status. We did not see a difference in the association with premenopausal compared with postmenopausal use of "any" genital powder use, which is not consistent with a recent report (15) where an association with premenopausal use but not postmenopausal use was found. However, consistent with this report, we found a stronger association between "any" genital powder use and EOC among postmenopausal women who reported HT use compared with nonusers. This finding is also consistent with experimental data showing that in the presence of estrogen and/or estrogen and progesterone, the ability of macrophages to clear inert particulates is altered, enhancing the inflammatory response leading to the development of asthma in mouse offspring (20). It has also been proposed that chronic inflammation, resulting from exposure to body powder, whether through inhalation or through a transvaginal route, may exert a suppressive effect on adaptive immunity, leading to increased risk of EOC (30). These findings suggest that AA women may be particularly susceptible to exposure to body powder due to having higher endogenous estrogen levels compared with white women (31, 32). Because of the limited sample size, we were not able to evaluate associations with the timing or duration of HT use or the concurrent effects of both HT and powder use. Tests for interaction of the associations in the stratified analyses by HT use were not significant and our findings should be considered exploratory.

The results of the current study showed that genital powder use was associated with ovarian cancer risk in AA women and are consistent with localized chronic inflammation in the ovary due to particulates that travel through a direct transvaginal route. The dose–response observed for duration of genital powder use provides further evidence for the relationship between genital powder and overall EOC risk. Our data suggest that the increased risk due to use of genital powder applies to both serous and nonserous histologic subtypes of EOC. Use of "only" nongenital powder was not found to be associated with the serous subtype, but our data suggest a relationship with nonserous EOC. The association with serous EOC is consistent with several previous studies (4, 6, 14–17). Only the pooled analysis found associations with the endometrioid and clear cell subtypes (6). The association with any occupational talc exposure and EOC (OR = 1.31; data not shown), though not statistically significant, is also consistent with the results for "only" nongenital powder use and suggest other routes of exposure, aside transvaginal, may effect EOC risk.

A recent publication of data from the WHI, which did not find an association with genital talc use and ovarian cancer (18), was accompanied by an editorial that emphasized the challenges in assessing the exposure to talc due to the reliance on self-report (33). This limitation in the measurement of the exposure variables in the current study needs to be considered when interpreting our results. The possibility of differential misclassification exists in a case–control study such as AACES, especially due to heightened awareness of the exposure as a result of two recent class action lawsuits (21). Because of such publicity, we adjusted for date of interview in the analysis. However, there is still a possibility that recall bias may have caused some inflation of the ORs. Although our findings suggest that the publicity of the class action lawsuits may have resulted in increased reporting of body powder use, our data do not support that recall bias alone before 2014 versus 2014 or later would account for the associations with body powder use and EOC. It is possible that the lawsuits sharpened memories of body powder use and improved the accuracy of reported use for both cases and controls interviewed in 2014 or later. As the association with nongenital body powder use is not consistent with the published literature, the possibility of misclassification of exposure, residual confounding, or a chance finding cannot be ruled out as an explanation for the associations with nongenital powder use.

In summary, we found that the application of genital powder is associated with serous and nonserous EOC in AA women, a novel observation in this population that is consistent with some large studies in whites. Our data are consistent with the notion that localized chronic inflammation in the ovary caused by exposure to genital powder contributes to the development of EOC. Although associations with nongenital powder use and EOC have not been previously reported, we cannot rule out the possibility that this relationship may be specific to AA women. The high prevalence of exposure to both genital and nongenital body powder among AA women compared with the mostly white subjects (41%), as in the large pooled analysis (6), underscores the importance of the study's findings. The results of the current study suggest that the use of body powder is an especially important modifiable risk factor for EOC in AA women.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.M. Schildkraut, S.E. Abbott, M.L. Bondy, M.L. Cote, L.C. Peres, E.S. Peters, A.G. Schwartz, F. Camacho, F. Wang, P.G. Moorman
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Acknowledgments

The authors thank the AACES interviewers, Christine Bard, LaTonda Briggs, Whitney Franz (North Carolina), and Robin Gold (Detroit). The authors also thank the individuals responsible for facilitating case ascertainment across the ten sites including: Jennifer Burczyk-Brown (Alabama); Rana Bayakly, Vicki Bennett and Judy Andrews (Georgia); the Louisiana Tumor Registry; Lisa Paddock, Natalia Herman, and Manisha Narang (New Jersey); Diana Slone,

Yingli Wolinsky, Steven Waggoner, Anne Heugel, Nancy Fusco, Kelly Ferguson, Peter Rose, Deb Strater, Taryn Ferber, Donna White, Lynn Borzi, Eric Jenison, Nairmeen Haller, Debbie Thomas, Vivian von Gruenigen, Michele McCarroll, Joyce Neading, John Geisler, Stephanie Smiddy, David Cohn, Michele Vaughan, Luis Vaccarello, Elayna Freese, James Pavelka, Pam Plummer, William Nahhas, Ellen Cato, John Moroney, Mark Wysong, Tonia Combs, Marci Bowling, Brandon Fletcher, (Ohio); Susan Bolick, Donna Acosta, Catherine Flanagan (South Carolina); Martin Whiteside (Tennessee) and Georgina Armstrong and the Texas Registry, Cancer Epidemiology and Surveillance Branch, Department of State Health Services.

Grant Support

This work was supported by the NCI (grant number R01CA142081; to J.M. Schildkraut, A.J. Alberg, E.V. Bandera, J. Barnholtz-Sloan, M.L. Bondy, M.L. Cote, E. Funkhouser, E.S. Peters, A.G. Schwartz, P. Terry, and P.G. Moorman). Additional support was provided by the Metropolitan Detroit Cancer Surveillance System with funding from the NCI, NIH, and the Department of Health and Human Services (contract number HHSN261201000028C), and the Epidemiology Research Core, supported in part by the NCI Center (grant number P30CA22453; to A.G. Schwartz and M.L. Cote) to the Karmanos Cancer Institute, Wayne State University School of Medicine.

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Received December 14, 2015; revised May 2, 2016; accepted May 4, 2016; published OnlineFirst May 12, 2016.

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Cancer Epidemiology, Biomarkers & Prevention

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Association between Body Powder Use and Ovarian Cancer: The African American Cancer Epidemiology Study (AACES)

Joellen M. Schildkraut, Sarah E. Abbott, Anthony J. Alberg, et al.

Cancer Epidemiol Biomarkers Prev 2016;25:1411-1417. Published OnlineFirst May 12, 2016.

Exhibit F

Although relatively uncommon, some women had substantial lifetime risk based on reported risk factors and lack of behaviorally modifiable choices.

IGCS-0015
Ovarian Cancer

DOES TALC EXPOSURE CAUSE OVARIAN CANCER?
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Background and Aims:
Controversy surrounds the question of whether talc use causes ovarian cancer.

Design:
Formal systematic analysis of talc use and ovarian cancer.

Methods:
All accumulated epidemiologic evidence (23 case-control studies, 5 meta-analyses, and 3 analyses of a single cohort) and basic science studies were reviewed and graded for quality. Data were considered overall and by histologic subtype. Attributable Risk estimates were calculated. Factors favoring causality were the well-accepted Hill's criteria.

Results:
Talc use increased ovarian cancer risk by 30-60% in almost all well-designed studies. The Attributable Risk was 29%, meaning that elimination of talc use could protect more than one quarter or more of women who develop ovarian cancer. Risk elevations were found consistently among good case-control studies, 2 of 3 cohort analyses, and all meta-analyses/pooled analyses. Well-designed studies that considered dose-response by both duration and frequency all found higher risk among women exposed to more applications. A plausible biologic mechanism is inflammation, known to cause other epithelial cancers. The talc association is more specific to serous ovarian cancer. Systematic bias is excluded because talc use is a durable behavior unlikely to be subject to recall bias; good case-control studies were all population-based or cohorts averting selection bias; and multiple adjustment for other risk factors limited confounding.

Conclusion:
Hill's tenets suggest that talc use causes ovarian cancer. Several, but not all, baby powder manufacturers have already replaced talc with corn starch.

Exhibit G

Systematic Review and Meta-Analysis of the Association between Perineal Use of Talc and Risk of Ovarian Cancer

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Abstract

Over the past four decades, there has been increasing concern that perineal use of talc powder, a commonly used personal care product, might be associated with an increased risk of ovarian cancer.

Objectives: To systematically review all available human epidemiological data on the relationship between perineal use of talc powder and ovarian cancer, with consideration of other relevant experimental evidence.

Methodology: We identified 30 human studies for qualitative assessment of evidence, including 27 that were retained for further quantitative analysis.

Results: A positive association between perineal use of talc powder and ovarian cancer was found [OR: 1.28 (95% CI: 1.20 - 1.37)]. A significant risk was noted in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and in post-menopausal women receiving hormonal therapy. A negative association was noted with tubal ligation.

Conclusion: Perineal use of talc powder is a possible cause of human ovarian cancer.

Keywords: Talc; ovarian cancer; perineal; epidemiological studies; systematic review; meta-analysis; toxicological studies.

1. Introduction

Ovarian cancer is a common gynecologic cancer among women in developed countries, occurring at low rates among young women but increasing with age [1]. The annual incidence rate of ovarian cancer during the period 2005 – 2009 was 12.7/100,000 women, varying by ethnicity. The majority of ovarian cancers are diagnosed at an advanced stage, with 61% having distant metastases at diagnosis. Hereditary risk factors for ovarian cancer, specifically BRCA1 gene mutations, increase the risk above 35 years of age by about 2-3%.

In recent decades, there has been increasing concern that perineal exposure to talc, a commonly used personal care product, might be associated with an increased risk of ovarian cancer. However, the data describing this association is somewhat inconsistent. Perineal application of talc among women varies by geographic location (Supplementary Material I), with prevalence of use generally higher in Canada, the US and the UK compared to Greece, China and Israel [2].

In order to better characterize the potential ovarian cancer risk associated with perineal use of talc, we conducted a systematic review and meta-analysis of peer-reviewed human studies on this issue. We also examined additional in-vitro or in-vivo toxicological studies, which shed light on possible biological mechanisms that might support an association between and ovarian cancer.

2. Materials and Methods

2.1. Literature Search and Identification of Relevant Human Studies

A comprehensive, multi-step search strategy was used to to identify relevant studies on talc from multiple bibliographic databases, relevant national and international agencies and other grey literature sources (Supplementary Material II). Specifically, conducted a systematic search for all original studies involving human subjects that examined the association of genital/perineal use of talc powder and risk of ovarian cancer, including studies identified in a previous review by Berge et al. [3]. This review followed the PRISMA guidelines, and more specific guidance provided by the Cochrane Collaboration [4] (see Supplementary Material II for details).

Included studies were individually evaluated and scored by two reviewers (MT and NF), as detailed in the Table 1 and Supplementary Material XI. Studies included in previous reviews by both Berge et al. [3] and Penninkilampi et al [5] are compared in Supplementary Material I.

The quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) [6], as detailed in Supplementary Material IV. We used a cut-off point of 7+ stars to represent studies of higher quality.

2.2. Literature Search and Identification of Relevant Non-Human Studies

We conducted a (non-systematic) review of relevant non-human studies identified in three major bibliographic databases to identify potentially relevant animal

and in vitro studies (Supplementary Material V). Only studies that focused on perineal exposure to talc powder were included. For outcomes, studies that focused on any type of cancer including ovarian cancer and perineal exposure were considered. All retrieved studies were examined for relevance, reliability and overall quality using the Klimisch scoring system [7, 8] (Supplementary Material VII, VIII and IX).

Studies are classified into one of the following four categories of reliability: 1) reliable without restriction, 2) reliable with restrictions, 3) not reliable and 4) not assignable. Additionally, category (5) is assigned to special studies focusing on pharmacologic or mechanistic investigations.

2.3. Hazard Characterization

Epidemiological studies included in the systematic review were qualitatively assessed to examine their potential to inform a weight of evidence analysis. Findings from these studies were evaluated with respect to study design, exposure and outcome ascertainment, as well as potential sources of bias and confounding.

Animal studies were evaluated for evidence on the association between perineal application of talc and ovarian cancer. Additional information on mechanism of action and toxicokinetics derived from in-vitro and in-vivo studies was used in evaluating biological plausibility.

We evaluated the overall weight of scientific evidence by performing a qualitative evaluation of the findings collected from epidemiological studies as well as non-human studies, using the Hill criteria [9].

2.4. Quantitative Meta-Analysis

We conducted a meta-analysis of the risk of ovarian cancer in relation to perineal use of talc using quantitative risk estimates reported in 27 original studies, comprising three cohort studies and twenty-four case-control studies (included in Table 1). Studies that had analyzed overlapping study populations were assessed on a case-by-case basis for inclusion into the meta-analysis. The level of detail in the reported findings, including sample size and publication date, were considered when deciding which study to include in the case of overlap (Supplementary Material XIV).

Maximally adjusted odds ratios (ORs), hazard ratios (HRs) or relative risks (RRs) – measures that are largely comparable because of the relatively low rate of occurrence of ovariaion cancer – were extracted from the original studies. Details of the meta-analytic methods are provided in Supplementary Material XIV.

114 **Table 1: Characteristics and overall findings of all included studies (N=30).**

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Case-control studies						
Booth et al.* (1989), UK [10]	235/451	Range: 20-65 Mean: 52.4 (cases); 51.4 (controls)	Frequency	No trend found	Possible association with >weekly use.	5
Chang and Risch (1997), Canada [11]	450/564	Range: 35-79 Mean: 57.2 (cases); 57.5 (controls)	Ever use Frequency Duration Time of use Type of use	Possible exposure-response with frequency and duration of use	Positive association	7

[1] Newcastle-Ottawa Scale (NOS) score for each of the listed studies as assessed in our review

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Pelvic surgery Histology			
Chen et al.* (1992), China [12]	112/224	Mean: 48.5 (cases); 49.0 (controls)	Ever use;	No trend analysis conducted	Positive association with use >3 months	6
Cook et al. (1997), USA [13]	313/422	Range: 20-79	Ever use Duration Type of use Histology Lifetime applications	No trend found	Positive association.	7
Cramer et al. (1982), USA [14]	215/215	Range: 18-80 Mean ± SD: 53.2 ± 1.0 (cases); 53.5 ± 1.0 (controls)	Ever use Type of use Pelvic surgery	No trend analysis conducted	Positive association	6

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Cramer et al. (2016), USA [15]	2,041/2,100	Range: 18-80	Ever use; Frequency; Duration; Type of use; Histology; Type of powder; Pelvic surgery; Ethnicity; Age at first use; Time since last exposure;	Significant trend for years since exposure, frequency and duration of use, and number of lifetime applications	Positive association	7
Gates et al. (2008), USA [16]	New England Case Control (NECC): 1,175/1,202 Nurses' Health	Mean ± SD: 51 ±13 (NECC); Mean ± SD: 51 ±8 (NHS)	Ever use; Frequency;	Significant trend for frequency of use	Positive association	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
	Study (NHS): 210/600					
Godard et al. (1998), Canada [17]	153/152	Mean: 53.7	Ever use; Sporadic/familial	No trend analysis conducted	No association	5
Green et al. (1997), Australia [18]	824/860	Range: 18-79	Ever use; Pelvic surgery;	No trend found	Positive association	7
Harlow et al. (1989), USA [19]	116/158	Range: 20-79	Ever use; Type of use; Type of powder;	No trend analysis conducted	No association	7
Harlow et al. (1992), USA [20]	235/239	Range: 18-76	Ever use; Frequency; Duration; Type of use;	Significant trend for monthly frequency of use	Positive associations in certain subgroups (talc used before 1960, women <50	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Method of use; Histology; Tumor grade; Type of powder; Lifetime applications; Age of first use; Pelvic surgery;		years old, women with 1 or 2 live births)	
Hartge et al. (1983), USA [21]	135/171	Mean: 52.1 (cases); 52.2 (controls)	Ever use;	No trend analysis conducted	No association	5
Kurta et al. (2012), USA [22]	902/1,802	Range: No range reported (age 25+)	Ever use;	No trend analysis conducted	Positive association	6
Langseth & Kjaerheim (2004), Norway [23]	46/179	Not reported	Ever use,	No trend analysis conducted	No association	4

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Merritt et al. (2008), Australia [24]	1,576/1,509	Range: 18-79 Mean: 57.8 (cases); 56.4 (controls)	Ever use; Duration; Histology; Pelvic surgery; Age at diagnosis;	No trend found	Positive association strongest for serous and endometrioid subtypes.	7
Mills et al. (2004), USA [25]	249/1,105	Mean ± SD: 56.6 (cases); 55 (controls)	Ever use; Frequency; Duration; Year of first use; Histology; Pelvic surgery; Time of use; Tumor behavior; Cumulative use;	No trend found	Positive association for invasive and serous invasive tumors.	6

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Moorman et al. (2009), USA [26]	African-American: 143/189; White 943/868	Range: 20-74	Ever use; Ethnicity;	No trend analysis conducted	No association	6
Ness et al. (2000), USA [27]	767/1,367	Range: 20-69	Ever use; Duration; Method of use;	No trend found	Positive association for any method of use.	6
Rosenblatt et al. (1992), USA [28]	77/46 (analyzed)	Range: ≤30 – 80≥	Ever use; Duration; Type of use; Pelvic surgery;	Positive trend for duration of use since tubal ligation	Possible association	4
Rosenblatt et al. (2011), USA [29]	812/1,313	Range: 35-74	Ever use; Lifetime number of applications; Duration;	No trend found	Possible association	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Year of first use; Age of first use; Age of last use; Time of use; Type of use; Histology;			
Schildkraut et al. (2016), USA [30]	584/745	Range: 20-79	Ever use; Frequency; Duration; Histology; Lifetime applications; Menopausal status;	Significant trend with frequency and duration of use, and number of lifetime applications	Positive association	8
Tzonou et al. (1993), Greece [31]	189/200	Range: <70	Ever use;	No trend analysis conducted	No association	5

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Whittemore et al. (1988), USA [32]	188/539	Range: 18-74	Ever use; Frequency; Duration; Type of use; Pelvic surgery;	No trend found	Could neither implicate nor exonerate talc as an ovarian carcinogen	4
Wong et al. (1999, 2009), USA [33, 34]	462/693	Mean: 54.9	Ever use; Type of use; Duration; Pelvic surgery;	No trend found	No association	4
Wu et al. (2015), USA [35]	1,701/2,391	Range: 18-79	Ever use; Ethnicity;	No trend analysis conducted	Positive association among Hispanics and non-Hispanic whites, but not African Americans.	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Wu et al. (2009), USA [34]	609/688	Range: 18-74	Ever use; Frequency; Duration; Type of use; Histology; Time of use; Cancer stage;	Significant trend for frequency and duration of use, and number of lifetime applications	Positive association	7
Cohort studies						
Gates et al. (2010)*, USA [36]	797/108,870	Range: 30-55	≥/week vs <1/week; Histology;	No trend analysis conducted	Possible association that varies by histological subtype. No association with mucinous tumors.	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Gertig et al. (2000), USA [37]	307/78,630	Range: 30-55 (at cohort entry)	Ever use; Frequency; Histology; Race;	No trend found	Possible association (modest increase for serous invasive subtype)	5
Gonzalez et al. (2016), USA [38]	154/41,654	Range: 35-74 Median: 57.8	Ever use; Time of use;	No trend analysis conducted	No association	6
Houghton et al. (2014), USA [39]	429/61,285	Range: 50-79 Mean: 63.3	Ever use; Duration; Type of use; Histology;	No trend found	No association	7

** Study assessed for qualitative evidence but not included in the meta-analysis*

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3. Results

3.1. Evidence from Human Studies

The multiple database search for original human studies yielded 656 references. Although grey literature search yielded another 477 references, only 5 were judged relevant the present analysis. Automatic followed by manual removal of duplicates identified 282 references for screening and review.

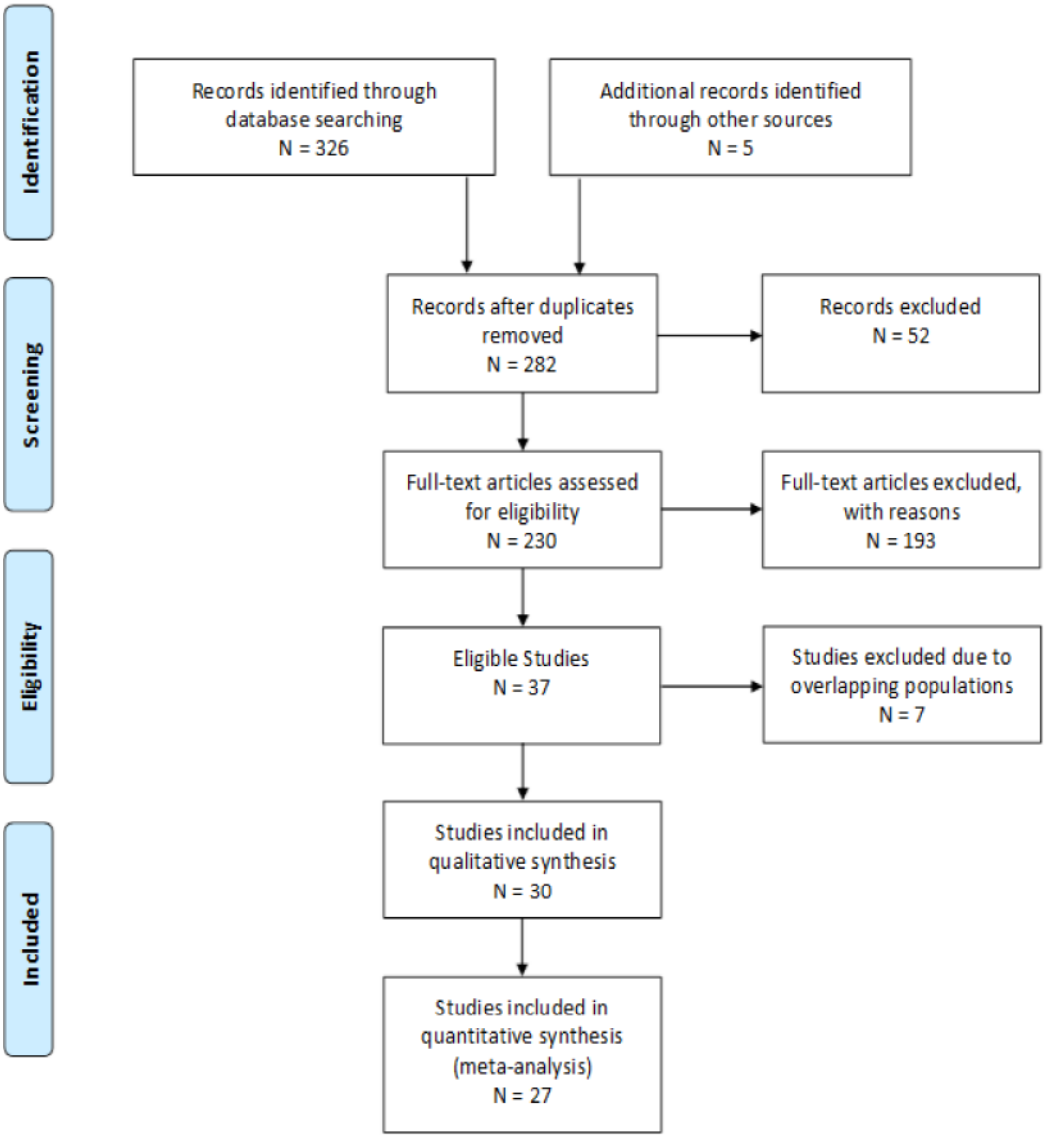
Multi-level screening and full-text examination resulted in the in the inclusion of 30 studies for further qualitative/quantitative analyses (Supplementary Materials X and XI). A detailed PRISMA flow diagram is shown in Figure 1 [40]. Key characteristics of the included 26 case-control studies and four cohort studies are summarized in Table 1.

Twenty-one of the thirty studies were carried out in the USA, with the remaining studies conducted in Europe (n=4), Canada (n=2), Australia (n=2) and China (n=1). Forty percent (n=12) of the studies were relatively recent, published in the last decade, with the remaining studies published between 1982 and 2006. The study populations generally included adult women. Several studies analyzed data from populations initially recruited for other purposes, such as the Nurses' Health Study (NHS) [15, 36, 37] and Women's Health Initiative (WHI) [39].

The number of ovarian cancer patients analyzed varied from as few as 46 cases [23] to 22,041 cases [15]. Twenty-seven out of the 30 included studies assessed the association between ever use of perineal talc use and ovarian cancer. Subgroup

136 analyses examining the effect of frequency and duration of use, type of use, period of

137 use and other factors varied among these studies (Table 1).



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139 Figure 1: PRISMA Flow Diagram

140 Sixty three percent (n=19) of the studies concluded the presence of a positive

141 association between perineal exposure to talc powder and ovarian cancer risk [10-16,

18, 20, 22, 24, 25, 27-30, 34-36]. Ten studies concluded the absence of an association [17, 19, 21, 23, 26, 31, 33, 37-39]. Only one study could not reach a clear conclusion on the presence or absence of an association [32]. Many of the included studies reported variability in some of the analyzed subgroups regarding possible association between exposure to talk powder and risk of ovarian cancer. Supplementary Material X presents the findings and details of all the studies included in the analysis, while Supplementary Material XI summarizes the strengths and limitations of each of these studies as identified by the original study authors and by us.

3.2. Evidence from Non-Human studies

After removal of duplicates, the bibliographic database searches on non-human studies initially yielded 1,165 references. The 51 retained animal studies focusing on the carcinogenicity of talc, mechanism of action, and toxicokinetics are summarized in Supplementary Material XII.

3.3. Hazard Characterization

3.3.1. Evidence from Human Studies

The case-control studies generally included adult women participants. Cases were commonly selected from registries or hospital records, and included all eligible subjects within a specific geographic region and diagnosed with ovarian cancer within a predetermined time period. Controls were generally matched to cases by age and residence. All the included studies compared the risk of ovarian cancer in ever vs never

users of talc (perineal application). However, several of the studies also included subgroup analyses to examine the potential effect of frequency of use, duration of use, tumor histology, ethnicity, method of use, lifetime number of applications, year of first use, and menopausal status. Some authors concluded that the risk of ovarian cancer is limited to [or stronger in] certain subgroups (weekly talc users, premenopausal women) or for specific histology types (notably serous tumors).

Studies reported effect estimates adjusted for a variety of potential confounders (see detailed tables in Supplementary Material X & XI). Age and parity were considered the two most important variables that could introduce potential bias, based on prior literature: few studies reported findings that were not adjusted for these two variables. As many of the studies only reported on the ovarian cancer risk assessing only one exposure category (comparing only ever vs never users of talc), exposure-response analyses were not done in all studies. When conducted, findings from trend analyses were not consistent.

3.3.2. Evidence from Non-Human Studies

The following aspects were considered in the weight of evidence assessment of ovarian cancer and perineal exposure to talc:

- hazards arising from the physical and chemical properties of talc, including potential structure-activity relationship indicative of carcinogenic potential;
- the toxicokinetics of talc and the ability to migrate from the perineal area to ovaries and quantity at the actual target site (the tissue dose);

- evidence on ovarian cancer reported in animal studies; and
- findings from in vitro studies suggestive of mechanism of action of carcinogenic effect.

While the data from the animal studies considered various routes of talc administration are inconsistent [41-46], there are observations from in vivo and in vitro studies which support the potential for local carcinogenic action of talc on fallopian, ovarian and peritoneal epithelium [27, 47-53].

The results from the *in vitro* studies are informative for mechanisms of action of possible carcinogenicity. Smith and colleagues [54] identified 10 key characteristics (KCs) commonly exhibited by established human carcinogens.

Oxidative stress (KC 6) and inflammation (KC 5) in cell cultures induced by talc have been reported by several authors [48], corresponding to two of the 10 key characteristics (KCs) described by Smith et al. [54]. Several authors suggested additional potential mechanisms of action through cell proliferation (KC 10) and changes in gene expression, presumably facilitated by oxidative stress and dysregulated antioxidant defense mechanisms [49, 55].

Chronic perineal or vaginal exposures of animals to talc do not directly affect ovulation or steroidal hormone levels, but can induce chronic local inflammation, which has been suggested as a risk factor for ovarian cancer [56]. Mechanism of action studies suggested that talc can complex iron on the surface and disrupt iron homeostasis, associated with oxidant generation, macrophage distress and leukotriene

released by macrophages in the surrounding cells resulting in the inflammatory response which could act as a tumor promoter in both animals and humans [48, 50, 51].

The changes seen in cultured cells after exposure to talc [50, 51] are consistent with those inflammatory and proliferative processes in the lungs seen in laboratory animals after inhalation exposure in a 1993 study conducted by the US National Toxicology Program [47]. In female rats, hyperplasia of alveolar epithelium was associated with inflammatory response and occurred in or near foci of inflammation [47]. The severity of the fibrous granulomatous inflammation in the lungs increased with increased talc concentrations and exposure duration and a significant association was observed between inflammation and fibrosis in the lungs and the incidence of pheochromocytomas in this study [47]. Overall, the avaialbe experimental data suggest irritation, followed by oxidative stress and inflammation, may play be involved in local carcinogenic effects of talc in the ovaries.

Local inflammation of the epithelial ovarian surface in rats following by injection of a suspension of talc particles demonstrated the development of foreign body granulomas surrounding talc particles and large ovarian bursal cysts [53]. It is generally accepted that benign and malignant ovarian epithelial tumors arise from surface epithelium and its cystic derivatives, and surface epithelial cysts have a greater propensity to undergo neoplasia than does the surface epithelium itself [57]. Evidence of neoplasms of epithelial origin, nuclear atypia, or mitotic activity in the surface epithelium was not found in this study; however, focal areas of papillary changes in the surface epithelium consistent with the histological signs of premalignancy were observed in 40% of treated animals [53].

Data on talc migration in the genital tract of animals is inconsistent, but could not exclude such possibility [58-61]. Some studies have reported lack of neutron-activated talc migration from the vagina to the ovaries in cynomolgus monkeys [58], but talc particles were identified in the ovaries of rats that received intrauterine instillation of talc [60]. Radioactivity was not found in the ovaries of rabbits dosed intravaginally with tritium-labelled talc, but was detected in cervix and fallopian tubes [59-61]. In studies in humans, Henderson and colleagues [62] examined tumor tissue of female patients with ovarian and cervical tumors. The authors detected talc particles in histological samples from 10 of 13 ovarian tumors, 12 of 21 cervical tumors and in 5 samples of 12 normal ovarian tissues [62].

Historically, the concern for talc carcinogenicity has been associated with its contamination by asbestos fibers (tremolite) [63], which is considered carcinogenic to humans [2]. Talc, including baby powder, available in the US, contains only U.S. Pharmacopeia (USP) grade pure talc [64]. Talcum powder has been asbestos-free since the 1976 where the specifications for cosmetic talc were developed [65].

3.3.3. Weight of evidence for carcinogenicity

Based on our evaluation of the weight of multiple lines of evidence, we concluded that perineal application of talc is a possible casue of cancer ovarian cancer in humans. In 2010 the Internatinal Agency for Research on Cancer [2] categorized perineal use of talc-based body powder (not containing asbestos or asbestiform fibers) as “possibly carcinogenic to humans (Group 2B)” [66].

Table 2 summarizes the available evidence for the association of ovarian cancer with perineal application of talc, organized around the nine Hill criteria [9]. Additoinal details of this evaluation are given in Supplementary Material XIII.

Table 2: Summary of evidence for each of the Hill Criteria of causation, as applied to perineal application of talc and ovarian cancer

Criterion	Summary of Evidence
Strength of association	• Out of the 30 epidemiological studies, six reported positive association of statistical significance with a risk value (relative risk or odds ratio) of 1.5 or greater • None of the cohort studies (n=3) found statistically significant association
Consistency	Fifteen out of thirty studies reported positive and significant associations reported in: • Different ethnicities (Caucasians, African Americans, and Latin Americans); • Over four decades (1982 - 2016); • Mostly in studies from the United States but also in other countries (Canada, Australia and China) • Case-control studies but not in cohort studies
Specificity	• Overall, the perineal talc exposure is specifically associated with cancer of the ovary and not other organs • No evidence of other target organs (e.g., liver) being associated with perineal application of talc (via systemic exposure)

Criterion	Summary of Evidence
	• Thirteen studies included analyses by histologic type of ovarian cancer, and eight of them found a significant increase in the risk of serous ovarian cancer in talc users
Temporality	• In all case-control studies reporting positive outcome, the participants recalled that exposure to talc preceded the reported outcome • In cohort studies, the follow up period could have been inadequate (<15 years) to detect a potential association between talc exposure and ovarian cancer
Biological gradient (exposure-response)	• About half of the epidemiological studies assessed only one level of talc exposure (ever vs never usage) • Of the 12 studies reporting a positive association, six studies found significant exposure-response trend, particularly with medium and high frequency usage groups Regarding duration of use/exposure to talc, several studies reported the greatest risk in the 20+ years of use exposure group, followed by the 10-20 years' group, then the <10 years' group
Biological plausibility	• Particles of talc appear to migrate into the pelvis and ovarian tissue causing irritation and inflammation • Transport of talc via perineal stroma and presence in ovaries documented • Chronic inflammatory response and alteration in local immunogenicity are possible mechanisms
Coherence	• Results from talc epidemiology studies are coherent with the current knowledge on the risk factors for ovarian cancer (e.g., factors/physiological states associated with greater frequency and duration of ovulation are associated with increased risk of ovarian cancer)

Criterion	Summary of Evidence
	• Many (but not all) case-control studies reported lower risk of ovarian cancer in women who underwent pelvic surgery or tubal ligation (which disrupts the pathway and movement of talc from lower to upper genital tract) & suppressed ovulation
Experimental evidence	• Perineal application of talc has not been tested in an animal model of ovarian cancer • The single animal cancer bioassay with talc conducted by the US National Toxicology Program was only by the inhalation route • Rodent models may be of limited relevance because of ovulations occurring only or mainly during the breeding season and the rarity of ovarian epithelial tumors in these animals and ovaries are variously enclosed in an ovarian bursa.
Analogy	• Talc and asbestos are both silicate minerals • Talc has been variably contaminated with asbestos (tremolite and anthophyllite; until 1976, talcum powders were only required to contain at least 90% mineral talc) • The pleural and peritoneal mesotheliomas caused by asbestos are histologically similar to epithelial ovarian cancer associated with talc • In animal models, asbestos induces ovarian epithelial hyperplasia similar to early epithelial tumors reported in women with past use of talc

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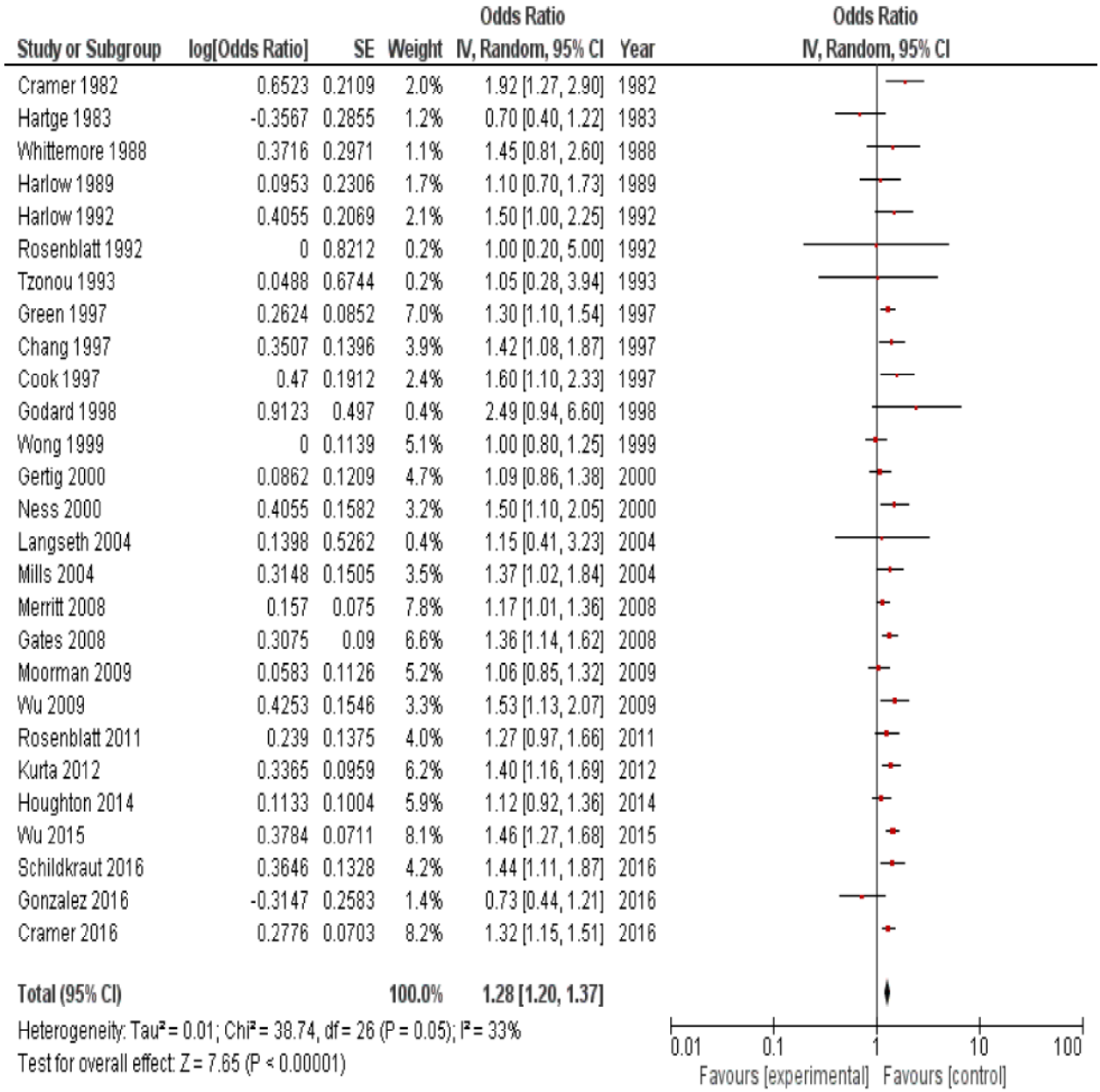
3.4. Meta-Analysis

The use of genital talc was associated with a significant increase in the risk of epithelial ovarian cancer, with an overall odds ratio [OR] based on our meta-analysis of 1.28 (95% confidence interval [CI]: 1.20 to 1.37 P<0.0001, I^2= 33%), as presented in

265 Figure 2. This result is comparable to those of earlier meta-analyses conducted by other

266 investigators [3, 5, 67-69] as shown in Supplementary Material I.



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268 **FIGURE 2: Forest plot of the meta-analysis results on perineal use of talc and**

269 **risk of ovarian cancer**

An increased risk is more apparent in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and post-menopausal women receiving hormonal therapy, as well as for the serous and endometrioid types of ovarian cancer (Table 3 and Supplementary Material XIV). A negative association was noted with tubal ligation. Our analysis pooled risk estimates from 27 original studies including 3 cohort studies and 24 case-control studies, spanning across four decades (1982-2016) and including a total of 16,352 cases and 19,808 controls from different ethnicities.

In assessing heterogeneity among included studies, most subgroup analyses reported an I^2 statistic ranging between 0%-40%, which will have only a minimal impact on the analysis [4]. Only three subgroup analyses (ethnicity, menopausal state, and pelvic surgery) reported an I^2 statistic of 77%-78%, where considerable heterogeneity might have had an impact on the results [4]. (See Table 3 and Supplementary Material XIV for a listing of I^2 statistic values for the different subgroup analyses)

Whereas case-control studies showed a significant increase in the risk of ovarian cancer for ever vs never users of talc powder [OR: 1.32 (95% CI: 1.24 to 1.40), P < 0.00001, I^2= 22%], cohort studies failed to show a significant increase in risk [OR: 1.06 (95% CI: 0.9 to 1.25), P= 0.49, I^2= 17%]. Thirteen out of 24 case-control studies (54%) showed a statistically significant association, whereas none of the 3 cohort studies showed a significant overall association between ever vs never genital talc exposure and risk of ovarian cancer.

Subgroup analysis by study quality (NOS≥7 vs NOS<7) did not show any significant differences in the overall pooled risk estimate. Similarly, there were no differences among subgroup analysis conducted by decade of publication. A significant association was observed for population-based studies [OR: 1.34 (95% CI: 1.27 to 1.41), P < 0.00001, I^2= 0%], but for enlisting hospital-based controls [OR: 0.96 (95% CI: 0.78 to 1.17), P= 0.66, I^2= 0%].

We conducted influence analysis to examine the impact of individual studies on the results of our meta-analysis. No appreciable changes were observed regarding the overall association of perineal talc exposure and the risk of ovarian cancer in response to the exclusion of any one study. Detailed results from the influence analysis are provided (Supplementary Material XIV).

Subgroup analysis based on ethnicity indicated that Hispanic women using talc showed the most significant increase in risk of ovarian cancer [OR: 1.70 (95% CI: 1.17 to 2.47), P = 0.005, I^2= 0%], followed by White women [OR: 1.28 (95% CI: 1.10 to 1.49], P= 0.001, I^2= 56%). African-American women showed a non-significant association with ovarian cancer in [OR: 1.67 (95% CI: 0.90 to 3.10), P= 0.1, I^2= 48%].

Analyzing exposure by frequency of talc use, talc exposure was stratified into three groups: high (once daily for >25 days/month), medium (once daily for 10–25 days/month) and low (once daily for 1–<10 days/month). The OR for the high-use group was higher in the high-use group compared to the other two groups (medium and low-use groups). Duration of talc use was stratified into three groups: <10 years, 10 – <20 years, and 20+ years. The overall odds ratio of the <10 years' group was lower than the

OR for the 10 – <20 years' group. On the other hand, the OR for the 20+ years' group was lower and not statistically significant. However, this OR was based on two studies that showed considerable heterogeneity (I^2=75%). Examining the method of application of talc, application to the underwear subgroup had a statistically significant OR, which was the highest among all subgroups. Diaphragm use showed an expected, yet non-significant, negative association with ovarian cancer, which may be due to its action blocking the ascent of talc particles up the reproductive tract.

Pooled risk estimates were statistically significant for two histological types of ovarian cancer: serous tumors [OR: 1.38 (95% CI: 1.22 to 1.56), P < 0.00001, I^2= 0%] and endometrioid tumors [OR: 1.39 (95% CI: 1.05 to 1.82), P= 0.03, I^2= 2%]. The mucinous type showed a non-significant association [OR: 1.05 (95% CI: 0.85 to 1.29), P= 0.41, I^2= 23%], while there were not sufficient studies to examine the other types of ovarian cancers. Regarding tumor behavior, there was no appreciable difference between invasive [OR: 1.38 (95% CI: 1.15 to 1.65), P= 0.0004, I^2= 0%] and borderline [OR: 1.43 (95% CI: 1.08 to 1.89), P= 0.01, I^2= 19%] grades of ovarian cancer. Borderline serous tumors showed slightly greater risk [OR: 1.39 (95% CI: 1.09 to 1.78), P= 0.008, I^2= 0%] compared to the serous invasive grade [OR: 1.32 (95% CI: 1.13 to 1.54), P= 0.0004, I^2= 24%], while both showed a significant association with perineal talc exposure. However, the mucinous tumors showed a non-significant association with talc exposure, with invasive grades being associated with a greater risk [OR: 1.34 (95% CI: 0.48 to 3.79), P= 0.58, I^2= 70%] compared to the borderline grade [OR: 1.18 (95% CI: 0.76 to 1.82), P < 0.46, I^2= 34%].

Among post-menopausal women, those receiving hormonal therapy showed the greatest risk [OR: 2.28 (95% CI: 1.72 to 3.01), P < 0.00001, I^2= 0%], followed by pre-menopausal women [OR: 1.42 (95% CI: 1.16 to 1.75), P= 0.0008, I^2= 0%], and then post-menopausal women not receiving hormonal therapy [OR: 1.05 (95% CI: 0.84 to 1.32), P= 0.66, I^2= 25%]. This subgroup analysis suggests that hormonal factors, especially estrogens influence the risk of developing ovarian cancer among postmenopausal women who have perineal talc exposure.

Women with prior ligation of the Fallopian tubes showed a significant reduction in risk [OR: 0.64 (95% CI: 0.45 to 0.92), P= 0.02, I^2= 19%] against ovarian cancer compared to hysterectomy [OR: 0.89 (95% CI: 0.54 to 1.46), P= 0.65, I^2= 61%], whereas both surgeries combined showed no effect [OR: 1.06 (95% CI: 0.78 to 1.42), P= 0.72, I^2= 61%]. This might be attributed to the fact that tubal ligation is usually performed at an earlier age, thus preventing entry of talc into the reproductive tract earlier and prolonged exposure to talc, compared to hysterectomy that is performed later in life where a higher exposure has already taken place. In a recent meta-analysis [70], the authors reported a negative association of tubal ligation (27 studies) and hysterectomy (15 studies) with the risk of ovarian cancer: this negative association was more apparent in women who had the surgery at an earlier age. A highly plausible mechanism for this association, as suggested by the authors, involves blocking of ascent of agents such as talc to the ovaries.

A summary of results of our meta-analysis is shown in Table 3. Forest plots of all sub-group analyses are provided in Supplementary Material XIV.

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359 **Table 3: Results of the subgroup analysis of talc exposure and ovarian cancer**

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
1. Talc use			
Ever vs. Never	27	1.28 [1.20, 1.37]	33% [< 0.00001]
Ethnicity	3		77% [0.08]
African Americans	3	1.67 [0.90, 3.10]	48% [0.10]
Hispanics	2	1.70 [1.17, 2.47]	0% [0.005]
Whites	3	1.28 [1.11, 1.49]	56% [0.001]
Asians	1	0.04 [0.01, 0.16]	N/A
2. Study Assessment			
2.1. Study Design	27		33% [< 0.00001]
Case-Control	24	1.32 [1.24, 1.40]	22% [< 0.00001]
Cohort	3	1.06 [0.90, 1.25]	17% [0.49]
2.2. Type of Controls	24		22% [< 0.00001]
Hospital-based	4	0.96 [0.78, 1.17]	0% [0.66]
Population-based	19	1.34 [1.27, 1.41]	0% [< 0.00001]
Combined	1	1.45 [0.81, 2.60]	N/A
2.3. Quality Score (NOS)	27		33% [< 0.00001]
NOS >=7	12	1.32 [1.25, 1.40]	0% [< 0.00001]
NOS <7	15	1.21 [1.05, 1.39]	47% [0.009]
2.4. Publication Year	27		33% [< 0.00001]
1980-1989	4	1.23 [0.81, 1.88]	66% [0.33]
1990-1999	8	1.30 [1.13, 1.50]	24% [0.0003]
2000-2009	8	1.25 [1.14, 1.37]	18% [< 0.00001]
2010 and beyond	7	1.31 [1.18, 1.45]	44% [< 0.00001]
3. Talc Exposure			
3.1. Frequency of Use	7		35% [< 0.00001]
Low	5	1.22 [0.96, 1.54]	54% [0.10]
Medium	2	1.22 [0.98, 1.53]	0% [0.08]
High	7	1.39 [1.22, 1.58]	23% [< 0.00001]
3.2. Duration of Use	6		5% [0.0008]
<10 Years	5	1.22 [1.03, 1.45]	0% [0.02]

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
10 - <20 Years	2	1.42 [1.02, 1.99]	0% [0.04]
20+ Years	2	1.19 [0.71, 1.98]	75% [0.51]
3.3. Method of Use	13		52% [0.001]
Sanitary Napkin	11	1.12 [0.91, 1.39]	50% [0.29]
Diaphragm	10	0.87 [0.72, 1.05]	25% [0.14]
Underwear	2	1.70 [1.27, 2.28]	0% [0.0004]
Male Condom	3	0.99 [0.73, 1.32]	0% [0.92]
4. Tumor Histology			
4.1. Tumor Histology	8		23% [< 0.00001]
Serous	7	1.38 [1.22, 1.56]	0% [< 0.00001]
Mucinous	5	1.05 [0.85, 1.29]	23% [0.41]
Endometrioid	6	1.39 [1.05, 1.82]	2% [0.03]
Clear Cell	1	0.63 [0.15, 2.65]	
5. Tumor Behavior			
5.1. All Grades	4		0% [< 0.00001]
All Invasive	3	1.38 [1.15, 1.65]	0% [0.0004]
All Borderline	4	1.43 [1.08, 1.89]	19% [0.01]
5.2. Serous	5		0% [< 0.00001]
Serous Invasive	5	1.32 [1.13, 1.54]	24% [0.00004]
Serous Borderline	3	1.39 [1.09, 1.78]	0% [0.008]
5.3. Mucinous	3		38% [0.40]
Mucinous Invasive	2	1.34 [0.48, 3.79]	70% [0.58]
Mucinous Borderline	3	1.18 [0.76, 1.82]	34% [0.46]
5.4. Endometrioid	1		N/A
Endometrioid Invasive	1	1.38 [1.06, 1.80]	
5.5. Clear Cell	1		N/A
Clear Cell Invasive	1	1.01 [0.65, 1.57]	
6. Modifiers			
6.1. Menopausal State	2		78% [0.007]
Pre-menopausal	2	1.42 [1.16, 1.75]	0% [0.0008]
Post-Menopausal (HT)	2	2.28 [1.72, 3.01]	0% [< 0.00001]
Post-Menopausal (no HT)	2	1.05 [0.84, 1.32]	25% [0.66]

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
6.2. Pelvic Surgery	7		78% [0.35]
Tubal Ligation	3	0.64 [0.45, 0.92]	19% [0.02]
Hysterectomy	4	0.89 [0.54, 1.46]	61% [0.65]
Combined	4	1.06 [0.78, 1.42]	61% [0.72]

*** NOS:** Newcastle-Ottawa Scale for quality scoring of observational studies

**** Low:** Once daily for 1 – <10 days/month; **Medium:** Once daily for 10 –25 days/month; **High:** Once daily for >25 days/month

3.5. Exposure-Response Assessment

The effect of increasing frequency or duration of perineal use of talc and the risk of ovarian cancer was assessed in the majority of the studies included in this review. Conflicting findings were reported on the nature of the exposure-response relationship: 11 studies concluded that there is no exposure-response, five studies reported a significant positive trend with either frequency or duration of talc use, and two studies concluded that there might be an exposure-response. The remaining twelve studies did not perform or report on trend analyses.

Findings from the seven studies that indicated a potential increased risk of ovarian cancer associated with increasing use of talc are presented in Table 4. The study by Cramer et al. [15] provides the strongest evidence of an exposure-response relationship and could be considered as a key study for exposure-response assessment. The data used in this study were generated from the Nurses' Health Study

originally conducted by Belanger et al. [71], a well-designed high quality cohort study of the factors affecting women's health. The results of this study show an increased risk of ovarian cancer at the three highest exposure categories in this study, with the risk at the lowest exposure level [OR: 1.15 (95% CI: 0.89 to 1.47)] being numerically, although not significantly, elevated. Other studies in Table 4 have provided findings in support of an exposure response based on increasing number of talc applications [20, 30, 34].

In order to permit more direct comparisons of the exposure-response findings from these studies, and whenever the original study data permits, we standardized exposure measurements into talc-years as shown in Figure 3. Data points were selected from studies after excluding potential data points that are lacking precise information on the level of exposure to talc. The mid-point of the exposure categories in the exposure-response studies was used for exposure-response assessment.

Overall, the graphical results shown in this Figure 3 suggest a possible increasing trend in ovarian cancer risk with increasing cumulative exposure to talc; however, there is also a high degree of uncertainty surrounding many of the individual risk estimates. (A formal statistical test for trend was not attempted because of the high degree of heterogeneity among studies noted previously in our meta-analysis discussed in section 3.4.)

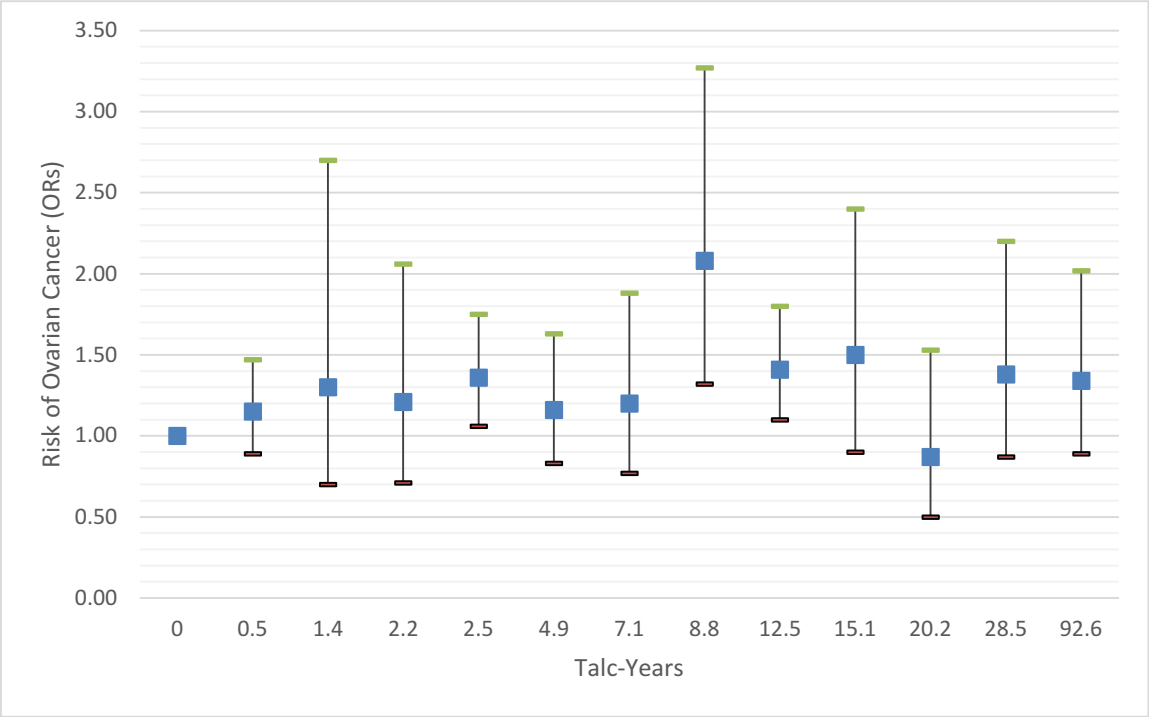

Figure 3: Ovarian cancer risk estimates at increasing levels of exposure to talc, as reported from multiple studies

405 **Table 4: Summary of studies that reported ORs for increasing number of lifetime perineal talc applications**

Study	Stratification	Reported Exposure-Response Strata	aOR*	95% CI
Schildkraut et al. (2016) [30]	Lifetime genital powder	<3,600 applications, any genital use vs (never use)	1.16	[0.83, 1.63]
		>3,600 applications, any genital use vs (never use)	1.67	[1.23, 2.26]
Whittemore et al. (1988) [32]	Overall trend	Overall trend for 30 uses per month	1.3	[0.88, 1.92]
Wu et al. (2009) [34]	By total times of talc use	≤ 5,200 times vs nonuse	1.2	[0.77, 1.88]
		5,201 – 15,600 times vs nonuse	1.38	[0.87, 2.20]
		15,601 – 52,000 times vs nonuse	1.34	[0.89, 2.02]
		> 52,000 times	1.99	[1.34, 2.96]
Mills et al. (2004) [25]	By cumulative use (frequency × duration)	First quartile (lowest exposure)	1.03	[0.59, 1.80]
		Second quartile	1.81	[1.10, 2.97]
		Third quartile	1.74	[1.11, 2.73]
		Fourth quartile (highest exposure)	1.06	[0.62, 1.83]
Rosenblatt et al. (2011) [29]	By lifetime number of applications of perineal powder after bathing	1-1,599 applications	1.21	[0.71, 2.06]
		1,600-4,799 applications	2.08	[1.32, 3.27]
		4,800-9,999 applications	0.87	[0.50, 1.53]
		≥10,000 applications	0.87	[0.48, 1.57]
Cramer et al. (2016) [15]	By total genital applications	≤360 total genital applications	1.15	[0.89, 1.47]
		361-1,800 total genital applications	1.36	[1.06, 1.75]
		1,801-7,200 total genital applications	1.41	[1.10, 1.80]
		>7,200 total genital applications	1.39	[1.11, 1.75]
Harlow et al. (1992) [20]	Total Lifetime Perineal Applications*	< 1,000 applications	1.3	[0.7, 2.7]
		1,000 - 10,000 applications	1.5	[0.9, 2.4]
		>10,000 applications	1.8	[1.0, 3.0]

406 * aOR: adjusted odds ratio

407 ** 10,000 applications are equivalent to daily use for 30 year

4. Discussion

The present analysis of the association between perineal use of talc powder and ovarian cancer risk considered four decades of scientific work exploring the epidemiological associations and non-human studies. The motivation for this review is based on two questions: what do human epidemiology studies of perineal talc exposure reveal about potential ovarian carcinogenicity, and what do in-vitro and in-vivo studies suggest about potential mechanisms of toxicity?

A systematic review of the human epidemiology studies was conducted to address the first question. Thirty observational epidemiologic studies were identified and assessed for quality using the NOS [6]. In parallel with the review of human epidemiological evidence, a (non-systematic) review of evidence exploring in vitro and in vivo toxicology data on talc was conducted to explore how talc might produce biological changes. This latter review provides some insights concerning possible mechanisms of talc toxicity, including oxidative stress, immune system alterations and inflammatory responses. However, it also indicates that talc is not genotoxic. In total, the epidemiology studies suggest that perineal exposure to talc powder is a possible human ovarian carcinogen but there are concerns that the actual exposure experienced by these women over the past 40-50 years is not well understood. As reported by Langesth and colleagues [67], there had been some concern that asbestos-contaminated talc powder that was produced prior to 1976 might have been a confounder; however, the similarity of findings between studies published prior to and after this point suggests asbestos contamination does not explain the positive association between perineal use of talc powder and risk of ovarian cancer [25, 27].

Human observational studies have inherent limitations that could bias the findings. Potentially important sources of bias reported in the included studies include: 1) selection bias due to low response rates from cases and controls or from limiting subjects to English-speaking women of two specific races, and 2) exposure misclassification due to recall bias inherent in case control studies. Other limitations included small sample sizes in some studies, small numbers of subjects in subgroup analyses, lack of information on duration of talc use in many studies that only compared ever vs never users, as well as lack of information on the talc content of the different brands of genital powders used. In two of the three cohort studies, the follow-up period between exposure assessment and end of study could have been inadequate to detect a potential association between talc exposure and ovarian cancer. Houghton et al. [39] reported a mean follow up of 12.4 years, while Gates et al. [36] followed a cohort of women for 24 years. However, Gertig et al. [37] and Gonzalez et al. [38] noted that one of their main limitations is the relatively short follow up periods that may not be adequate to detect a potential association between talc exposure and ovarian cancer. For example, studies of smoking and ovarian cancer suggest that follow-up periods as long as four decades improve recognition of the carcinogenic effects of smoking [72]; longer follow up periods may also improve characterization of the association between talc and ovarian cancer. In this regard, the minimum latency period for radiation-induced ovarian cancer among Hiroshima atomic bomb survivors has been reported to range from 15 to 20 years [73, 74]. Common strengths reported in most studies were the selection of population controls in many of the case control studies and having relatively large sample sizes that allowed a multitude of stratified analyses.

Effect estimates in this meta-analysis were pooled from 24 case control studies and 3 cohort studies, and refer to ever vs never use of perineal talc. Pooling by study design showed a notably higher risk estimate for case-control [OR: 1.32 (95% CI: 1.24 to 1.40), P < 0.00001, I^2= 22%] compared to cohort studies [OR: 1.06 (95% CI: 0.9 to 1.25), P= 0.49, I^2= 17%]. Although the reasons for this are unclear, the difference could potentially be due to issues relating to latency, study power, or exposure misclassification.

Although cohort study designs are efficient for examining diseases with a long latency period, it is essential that the period between talc exposure and the cancer diagnosis be sufficiently long. Gonzalez et al. [38] suggested that the latency period for ovarian cancer is between 15 to 20 years. In the cohort studies included in this review, Houghton et al. [39] reported a mean follow up of 12.4 years while Gates et al. [36] followed a cohort of women for 24 years. Gertig et al. [37] and Gonzalez et al. [38] noted that one of their studies' main limitations was the relatively short follow up periods that may not be adequate to detect a potential association between talc exposure and ovarian cancer.

In addition, cohort studies included may have been underpowered to detect an odds ratio (relative risk) of 1.3 estimated from the case control studies. This was noted by Narod et al. [75], who suggest that cohorts of at least 200,000 women would be needed to reach statistical significance if the true odds ratio is 1.3. The cohort studies included in this review included much smaller cohort sizes, ranging between 41,654 and 78,630 women.

Finally, in cohort studies, talc exposure was assessed at cohort entry and was used as a measure of chronic talc use during follow up. It is possible that women who were not exposed to perineal talc at the time of cohort entry began using talc at a later time, and vice versa, possibly introducing non-differential misclassification of exposure, which could bias the risk estimate towards the null value of unity. Conversely, in the presence of differential exposure misclassification, a bias away from the null hypothesis could accentuate differences between the cohort and case-control studies.

4.1. Exposures and outcomes

All epidemiological studies included in our review evaluated the association between the perineal application of talc and subsequent diagnosis of ovarian cancer. Perineal vs body exposure is an important distinction, as the movement of talc is thought to follow an ascending path from the perineum through the vagina, uterus and fallopian tubes to the ovarian (as well as fallopian tube and peritoneal) epithelium.

Ovarian cancer is a common gynecologic malignancy in developed and developing countries. Risk factors for ovarian cancer include age, infertility, nulligravidity, endometriosis, hereditary ovarian cancer, tobacco and asbestos.

Protective factors for ovarian cancer include oral contraceptives, bilateral tubal ligation, salpingo-oophorectomy, hysterectomy, and breast feeding [76]. It is a difficult cancer to diagnose early, with approximately 60% of the individuals diagnosed after the cancer has metastasized from the pelvic region, where this cancer begins. In the meta-analysis, comparing ovarian cancer risk among women who used talc versus those who

never used talc (using both case-control and cohort designs), we observed an approximate 30% increase in ovarian cancer risk in the group who used talc. The most common type of ovarian cancer seen in the general population, and among the women exposed to talc were of epithelial origin, most common histologic type (accounting for about 95% of all cases in the general population), and of serous morphology, the most common subtype (comprising about 75% in the general population).

The cell-type of origin and morphology of talc induced ovarian cancer is similar to that observed in typical ovarian cancer with approximately 95% derived from epithelium (from fallopian tube fimbriae, ovarian or peritoneal) with serous tumors as the most common subtype. Like most ovarian cancers, those associated with talc exposure are typically diagnosed late in the course of the disease (~60% are diagnosed after the disease has spread outside of the pelvis). This late diagnosis complicates our understanding of the history and origin of the disease.

Demographic factors were analyzed using subgroup analysis where possible, and these were generally consistent with what has been previously observed with respect to ethnicity and risk of ovarian cancer. Additionally, these data also provide support for a mechanism of ovarian cancer induction working via an inflammatory pathway associated with oxidative stress [27, 77, 78].

A small number of studies explored the issue of ethnicity: Asians (1 study), Hispanics (2 studies), and African-Americans and Whites (3 studies each). Among these studies the risk for talc associated ovarian cancer was 1.70 (Hispanics), 1.67 (African Americans), 1.28 (Whites) and 0.04 (Asians). These risk factors compare with the demographics of ovarian cancer in the US population with an overall prevalence of

ovarian cancer of 12.7/100,000 among Whites 13.4/100,00, Hispanics 11.3/100,000, African Americans 9.8/100,000, and Asians 9.8/100,000. The difference in US prevalence and risk of talc induced ovarian cancer among Hispanics and African Americans may provide further evidence concerning exposures or mechanism of action [76].

A variety of factors were assessed with respect to the studies contributing to the meta-analysis, including study quality (NOS) and publication year. In general, the risk of talc associated ovarian cancer was similar among studies with an NOS ≥7 or NOS <7. Year of publication also failed to demonstrate a significant impact on reported talc risk estimates.

4.2. Exposure metrics

Given that the epidemiological studies indicate that talc is a possible human carcinogen, we next evaluated the studies to identify those comparing differences in exposure. The initial assessment exploring frequency of use, utilized a qualitative exposure metric: low, medium and high. Ovarian cancer was observed to increase between the medium and high exposure groups, consistent with an exposure-response relationship. Several studies explored duration of use (years) and risk of ovarian cancer; 20+ years (2 studies),10 (5 studies), 10/20 (2 studies), and observed that the risk was greatest in the 20+ year exposure group, followed by lower risk in the 10/20 year and <10-year exposure groups.

Several studies explored the route of exposure or approach to talc application on ovarian cancer risk, including; hysterectomy, bilateral tubal ligation, diaphragm,

underwear, sanitary napkin, as these can provide insight into differences in exposure of the fallopian tube, ovarian and peritoneal epithelium. Use of a diaphragm, as well as tubal ligation act to interrupt exposure of perineal talc to reproductive tract. In contrast, application to underwear and sanitary napkin exposure will provide broader exposures. As hypothesized, the use of diaphragm and bilateral tubal ligation decreased ovarian cancer risk [22].

4.3. Modifying Factors

Modifiers of the risk of ovarian cancer, either associated with talc exposure, or a spontaneous disease, can provide clues to potential mechanisms of causation. Menopausal status and use of hormones can modify the risk for ovarian cancer. For example, among post-menopausal women receiving hormonal therapy the risk for ovarian cancer is greater than those who are premenopausal and those who are post-menopausal not receiving hormone therapy. It has also been observed that women receiving fertility treatment who do not become pregnant are at greater risk for ovarian cancer [22]. These data suggest that hormonal status (elevated estrogens and/or gonadotropins) plays a role in the mechanism of action of talc associated ovarian cancer.

Subgroup analyses in the meta-analysis indicated that interruption of the pathway from perineum to pelvis (as with bilateral tubal ligation or use of diaphragm) decreased risk for ovarian cancer. This supports the hypothesis that talc acts by local action on the ovary. Given the data developed in non-human studies suggesting an inflammatory response of epithelial cells to talc, and histological observations

567 corroborating those observations, additional support for an inflammatory pathway
568 leading to ovarian cancer is provided. One study recently explored the use of anti-
569 inflammatory drugs and observed a decreased risk for ovarian cancer, also supporting
570 the importance of an inflammatory pathway with oxidative stress [77].

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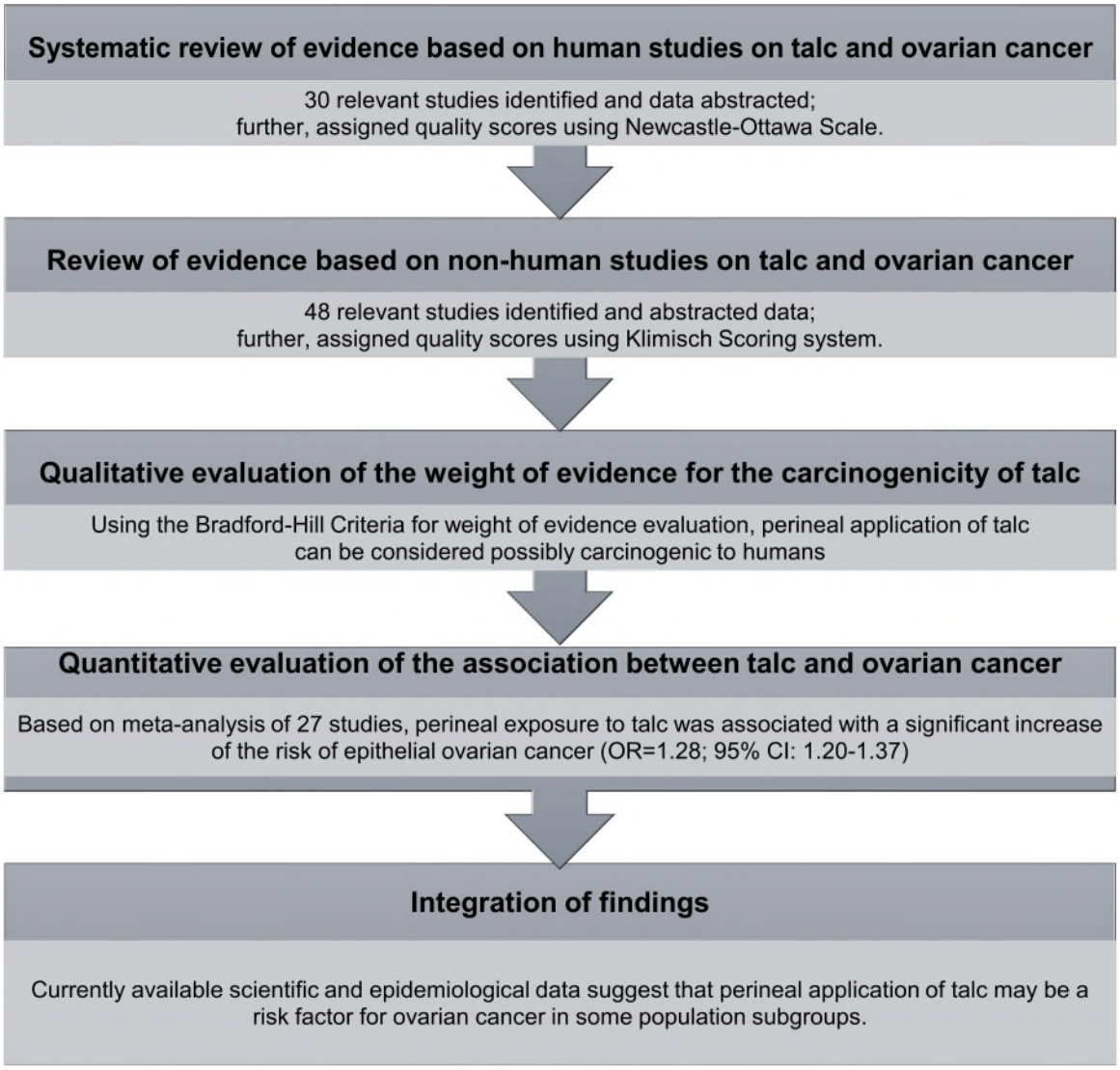

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573 **Figure 4: Detailed process flow for assessment of talc carcinogenicity**

574

5. Conclusion

We conducted an extensive search, examination, assessment and analysis of evidence from published human and non-human original as well as all published reviews that considered the association between genital/perineal use of talc powder and risk of ovarian cancer. The steps followed in conducting this review are summarized in Figure 4, along with the key findings at each step. Consistent with previous evaluations the IARC in 2010 [2], and subsequent evaluations by individual investigators [3, 5, 69], the present comprehensive evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.

6. Source of Funding

This work was supported by Health Canada as part of their Chemicals Management Plan via contract # 4600001163 to Risk Sciences International (RSI), Ottawa, ON, Canada.

7. Acknowledgments and Declarations

All authors who contributed to both this study and manuscript report no conflict of interest in relation to the planning for and conducting this study as well as the preparation of this manuscript. Although the research project and manuscript preparation were conducted under contract to Health Canada, the views and conclusions presented in this article are those of the authors alone.

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Exhibit H

Ultrastructural Pathology

ISSN: 0191-3123 (Print) 1521-0758 (Online) Journal homepage: https://www.tandfonline.com/loi/iusp20

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To cite this article: Sandra A. McDonald, Yuwei Fan, William R. Welch, Daniel W. Cramer, Rebecca C. Stearns, Liam Sheedy, Marshall Katler & John J. Godleski (2019): Correlative polarizing light and scanning electron microscopy for the assessment of talc in pelvic region lymph nodes, Ultrastructural Pathology, DOI: 10.1080/01913123.2019.1593271

To link to this article: https://doi.org/10.1080/01913123.2019.1593271

ULTRASTRUCTURAL PATHOLOGY
https://doi.org/10.1080/01913123.2019.1593271

Correlative polarizing light and scanning electron microscopy for the assessment of talc in pelvic region lymph nodes

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ABSTRACT

Perineal talc use is associated with ovarian carcinoma in many case-control studies. Such talc may migrate to pelvic organs and regional lymph nodes, with both clinical and legal significance. Our goal was to differentiate talc in pelvic lymph nodes due to exposure, versus contamination with talc in the laboratory. We studied 22 lymph nodes from ovarian tumor patients, some of which had documented talc exposure, to quantify talc using digestion of tissue taken from paraffin blocks and scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX). Talc particles correlated significantly with surface contamination assessments using polarized light microscopy. After adjusting for surface contamination, talc burdens in nodes correlated strongly with perineal talc use. In a separate group of lymph nodes, birefringent particles within the same plane of focus as the tissues in histological sections were highly correlated with talc particles within the tissue by *in situ* SEM/EDX ($r = 0.80$; $p < 0.0001$). We conclude that since talc can be a surface contaminant from tissue collection/preparation, digestion measurements may be influenced by contamination. Instead, because they preserve anatomic landmarks and permit identification of particles in cells and tissues, polarized light microscopy and *in situ* SEM/EDX are recommended to assess talc in lymph nodes.

ARTICLE HISTORY
Received 7 February 2019
Revised 1 March 2019
Accepted 6 March 2019

KEYWORDS/PHRASES
talc; scanning electron microscopy; carcinoma; birefringence

Introduction

In diseases related to foreign particulate exposure, accurate quantification of foreign material in tissue is important to document exposure and to correlate with disease occurrence or severity related to that tissue.[1] The issue is perhaps best appreciated for asbestos and pulmonary mesothelioma and fibrosis.[2] The most comprehensive quantification is obtained by digestion of a tissue sample, which uses much larger amounts of tissue that can be assessed in a histologic tissue section.[1] The procedure can be used to identify and quantify individual fibers by transmission electron microscopy (TEM) or scanning electron microscopy (SEM) and characterize them by energy dispersive x-ray analysis (EDX) to verify that their elemental signatures are compatible with a specific type of asbestos or other foreign material exposure[3] Application of TEM and/or SEM and EDX to tissue sections cut from paraffin blocks also provides quantification when the concentration of particles in tissue is sufficiently high.[4,5] This procedure may also show where the foreign material resides within a tissue section, such as exogenous particles localizing in macrophages within lymph nodes.[6] An estimate of foreign particulate exposure may also be obtained by studying histologic tissue sections under polarized light microscopy, which highlights birefringent material and its size and shape.[7,8] Besides the use of these methods in scientific studies to characterize exposures and disease, these techniques have also been used in medicolegal contexts related to claims of injury from various exposures, including asbestos.[1]

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One exposure of great current medical, public health, and medicolegal importance is the association of ovarian cancers with the use of talc cosmetic products in the genital area. Data related to this association come from epidemiologic studies which identified a clear excess of women with ovarian malignancy who had used talc in their genital area prior to developing cancer, compared to control women.[9–13] The International Agency for Research on Cancer has declared the use of talc (not containing asbestos) in the genital area as possibly carcinogenic (Class 2B) (IARC monograph, 2010).[14] The most recent summary of the epidemiologic data in 2018 found that genital talc use may increase the risk for ovarian carcinoma by about 30%.[15] Although the origin of the hypothesis about talc and ovarian cancer came, in part, from description of talc in ovarian tissue,[16] demonstration that talc is present in the ovarian tissue or the genital tract from women with ovarian cancer has not been a component of the epidemiologic studies, and published data regarding talc in women's pelvic organs is very limited. A study by Heller et al.[17] was done with digestion techniques followed by TEM on ovaries from 24 women having hysterectomy/oophorectomy for reasons other than ovarian malignancy. This study found talc in approximately half the samples, with no obvious correlation with genital talc use history, thereby suggesting to the authors that talc exposure may be relatively ubiquitous across the population. A subset of authors from the present study have previously described a case report[6] in which a woman with serous carcinoma of the ovary, and a history of talc usage in her genital area, was demonstrated to have talc in three of four examined pelvic lymph nodes.

In the study reported here, we assessed talc in a sizable set of lymph nodes of the pelvic region, representing multiple patients. Thus, we expanded on the lymph node analysis in the previous case report[6] as well as the study of non-malignant ovaries by Heller et al.[17] and we examined nodes in 22 patients with various types of ovarian tumors. We included the additional step of an independent polarized light microscopy study on the histological sections for each case; this procedure assessed the quantity and location of birefringent particles in relationship to tissue landmarks. By digesting the lymph node samples, assessing the presence of talc by SEM/EDX, and comparing that data to the findings by light microscopy, we assessed tissue surface contamination as a factor explaining the high talc burden in some cases, as opposed to talc that migrated to the nodes from perineal exposure. We also endeavored, by studying a separate group of lymph node cases, to show that polarized light microscopy is a useful adjunct to *in situ* SEM/EDX, since both preserve anatomic landmarks and can serve as indicators of talc whose source is not due to contamination.

Materials and methods

Twenty-two women with ovarian tumors who had received their care in 2004 and 2005 at the Brigham and Women's Hospital (BWH), and who had participated in larger epidemiologic studies of ovarian cancer in Eastern Massachusetts and New Hampshire, were selected for the study. Women in this series were selected consecutively on the basis of meeting eligibility criteria and not on the basis of whether they had used talc. To be eligible, cases must have had lymph nodes removed from the pelvic region as part of their surgery. Cases were ineligible if the only nodes available contained metastatic disease or if there was only one unaffected node available. Though most of the cases were malignant ovarian neoplasms, two cases (one a borderline tumor and the second a granulosa cell tumor) were included because the study's objective was focused on the quantification of talc in tissue and understanding contamination vs. exposure related findings. Relevant clinical data were available both from the medical record and questionnaires completed by the women that included information on the use of talc in the genital area or as a body powder. The study was approved by the BWH Institutional Review Board and the informed consent signed by the women included permission to study material removed at the time of surgery. This group of women had both digestion studies and light microscopic studies of their lymph nodes. For our purposes, nodes of interest included inguinal, iliac, and paraaortic, and potentially any node of the pelvic region used for sampling and/or staging in ovarian surgical oncology. In some cases, the

designation "pelvic lymph node" with laterality, but without further anatomic specification, was provided with a sample.

Talc is readily visible under polarizing light microscopy, where it may be found as both plates and fibrous forms, and where the particles or fibers are brightly birefringent and often in the size range 1–10 μm. Identification of talc by electron microscopy and energy-dispersive X-ray analysis (EDX), includes the plate-like particulate or fiber-like structure and a spectrum showing magnesium and silicon peaks within 5% of the theoretical atomic ratio of 0.75 and atomic weight percent ratio of 0.649.

For each patient case, we ascertained that an acceptable representative hematoxylin-eosin (H&E)-stained slide was available for the block prior to subsequent steps. Tissue was totally cut from the paraffin block with a cleaned scalpel, heat deparaffinized, and then multiple extractions were done with xylene to remove all residual paraffin. The tissue was weighed, then added to glass centrifuge tubes, and sodium hypochlorite solution was added for digestion over a 24–48 hr period. When digestion was complete, samples were centrifuged and the sediment resuspended in filtered distilled water and vortexed until no sediment was visible. The tubes were centrifuged again and the supernatant aspirated. Sediments were resuspended in 25% ethanol, mixed by vortexing and filtered through a 13 mm, 0.2 μm Millipore filter. Tubes were washed twice with 25% ethanol and filtered. Filters were dried in a desiccator and were mounted on a carbon planchette.

Samples were analyzed in a scanning electron microscope (Leo 1460VP) equipped with an EDX spectrometer (Oxford instruments with Inca software) or an Hitachi SU6600 field emission scanning electron microscope with Oxford EDX (Xmax 50SDD EDX detector) and Oxford instrumentation software (Aztec 3.3). At 2000x magnification, 200 particles or 100 random fields were analyzed for each case, whichever came first. Using various parameters, including the number of talc particles identified by their chemical composition, the area of each microscopic field times the number of fields examined, and the overall filter area, an estimate for the total number of talc particles in the specimen was calculated.

Because fat, fibrous tissue, and lymph node contributed to the weight of the material used for digestion and because there were differences in birefringent particle distribution patterns of the tissue surface, fat and fibrous tissue, and lymph node, a more accurate approach was needed by which we could estimate the contributions of the separate locations. Tissues on all slides were digitized. Using NIH Image J analysis software (an open source image processing program, www.imagej.com), the total areas (cm^2) of the tissue on the slides for each case were calculated, as well as the respective components of lymph node and fibroadipose (soft) tissue, with the sum of these areas adding up to the total tissue area. These figures were then multiplied by 0.25 cm (a typical thickness for tissue in paraffin cassettes from which the digested tissues were derived) to obtain total specimen volumes for the total tissue, and for the lymph node and soft tissue components. The total number of talc particles identified in the digestate by SEM was then divided by the total tissue volume to obtain the number of talc particles per unit volume (cm^3).

H&E slides of intact lymph node tissue corresponding to each digested paraffin sample were analyzed with an Olympus BH-2 light microscope equipped with polarizing filter capabilities (analyzer and rotating polarizer with specimen slide in between). Each slide was scanned systematically and completely at 200x magnification under polarized light. Slides typically contained one to several lymph node profiles with adherent fibroadipose tissue. Birefringent particles visually consistent with talc (typically 1–10 μm with birefringence) were counted that were located within the lymph node parenchyma and sinuses, and a separate count was made of particles in fibroadipose (soft) tissue, i.e. not within the lymph nodes proper. The counts of these two components were added to get the total count. Particles within fibroadipose tissue were counted only if they were at least one 400x (high-power) field away from the surface, so that obvious surface contamination was not included in the counts. The birefringent particles present within lymph nodes were taken to indicate clinically significant talc that migrated there through the lymphatic system. Birefringent particles on the physical surface of the tissues were not counted for these analyses but instead assessed as described below.

Using the aforementioned image analysis data which provided the areas (cm^2) for the total tissue on the slide as well as the lymph node and soft tissue components, for each slide, the respective tissue volumes were calculated by multiplying the areas by 4 μm (4×10^{-4} cm), a standard tissue section thickness on glass slides. The number of birefringent particles per unit volume were then calculated (through simple division) for each tissue component and for the overall tissue. This meant that the volume correction factor between tissue blocks and tissue slides was approximately 625 (0.25 cm thickness of tissue in blocks vs. 4 μm thickness of slides).

Additionally, for each of the 22 cases, a semi-quantitative visual estimate of surface contamination was made. This was done by observing the quantity and pattern of all polarizable material (typically birefringent particles of 1–10 μm, plus larger material such as paper, organic fibers, and other debris) that were present along the specimen edge and/or within one 400x (high power microscopic field) width from it. The objective here was to measure the degree to which the specimen surfaces might have been contaminated by physical manipulation during the acquisition and handling steps of the specimen in the Pathology department. Our estimate scores ranged from 0 to 3 and the criteria for the scoring was as follows (see Figure 1): 0, no polarizable material along surface; 1, occasional foreign particulates, rarely forming small clusters; 2, moderate numbers of surface particulates, forming occasional clusters or surface patches more numerous than in score 1; 3, frequent patches of particulates along with confluent stretches of contamination along the surface. Typically, such contamination was seen along the fibroadipose tissue surface with the nodal tissue interior to that. The contamination consisted typically of a mix of larger debris consistent with paper, along with smaller birefringent particulates similar to those seen and described in tissue sections (Figure 1). All contamination scores were done by a pathologist (JJG) in a blinded fashion (SEM and clinical data were unavailable at the time of scoring). A randomly chosen subset of the same cases was independently scored by a second pathologist (SM), also in a blinded fashion, to confirm successfully that the review standards agreed, and thus the scoring standards were being applied consistently.

Subsequent statistical analysis for the 22 cases was handled as follows: Talc counts were log transformed to create normal distributions. Spearman correlations were calculated to assess the relationship between potential contamination on the talc counts and each continuous variable, and partial correlations were used to examine the relationships between talc counts, adjusted for contamination. Linear regression was used to calculate crude and contamination-adjusted talc/total volume geometric means and 95% confidence intervals.

Also, as part of this report, we studied a second group of 19 lymph node specimens from 10 ovarian carcinoma cases. The 10 cases were consults of authors JJG and WW, which were de-identified, i.e. reported here without any patient identifiers, including the 18 recognized HIPAA identifiers.[18] All 19 tissue specimens had histologic slides and corresponding paraffin blocks available. In this component of the study, we assessed the relationship of the numbers of birefringent particles in the lymph node parenchyma in histological sections, and talc particles found by *in situ* SEM/EDX at deeper levels in the tissue blocks corresponding to those sections. Digestion was not performed on these cases; nor was information available on their talc exposure. Birefringent particles in the lymph nodes were exhaustively quantified by light microscopy as previously described (particles counted in respective lymph node and soft tissue components, added to a total count for each slide). The histologic slides typically contained from one to several lymph node profiles, each with adherent fibroadipose tissue. Counting was done without regard to the number of profiles; i.e. an aggregate count was obtained across all lymph node tissue on a slide.

The tissue blocks were handled with a procedure for *in situ* SEM/EDX distinct from the tissue digestion and filter analysis by SEM described in the previous component of the study. This *in situ* procedure was first described by Thakral and Abraham[4] for assessment of particulate materials in paraffin-embedded tissue. In the study reported here, the blocks were handled with particle-free gloves on pre-cleaned surfaces and sectioned removing ~30 micrometers of tissue

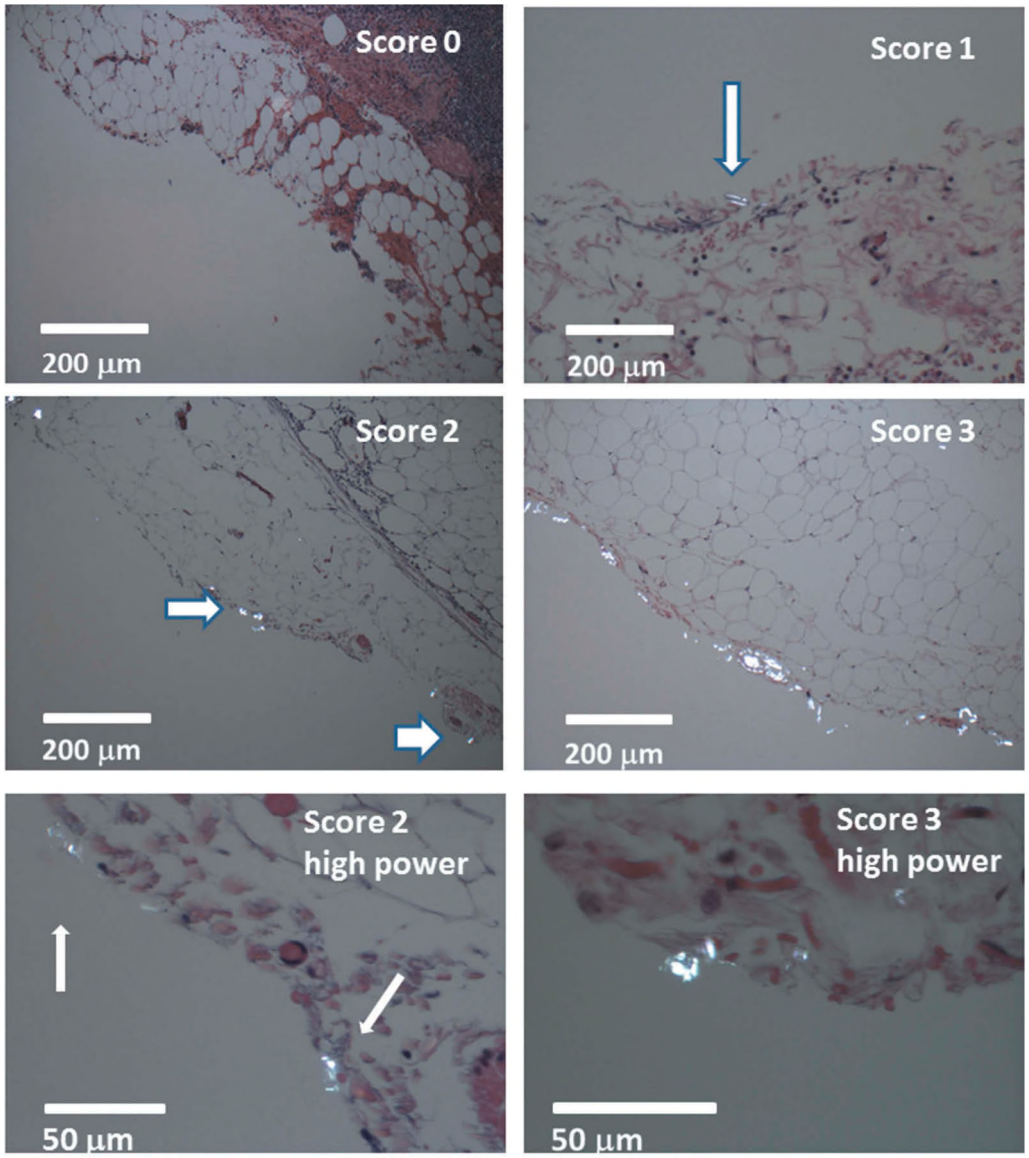

Figure 1. Tissue surface contamination score semi-quantitative grading. As shown especially in the two high-power images at bottom, the contamination material consisted typically of larger debris consistent with paper, along with smaller birefringent particulates. Surface contamination was typically found along the fibroadipose tissue surface, with lymph node tissue located underneath. Grading scheme is as follows: **Score 0**: no polarizable material along surface. **Score 1**: occasional birefringent particulates (arrows), rarely forming small clusters. **Score 2**: moderate numbers of surface birefringent particulates (arrows), forming occasional clusters or surface patches more numerous than in score 1. **Score 3**: frequent patches of particulates along with confluent stretches of contamination along the surface. (All images under polarizing light microscopy, H&E staining, all 100x except 400x [original magnification] in the bottom two images which respectively show score 2 and 3).

and paraffin using a rotary microtome with a new, clean stainless-steel blade. This sectioning was intended to remove any surface contamination from previous storage and handling. After the fresh surface was exposed, the block surfaces were washed in distilled, deionized water for 30 seconds to remove soluble surface materials such as sodium chloride and sodium phosphates used in processing for histology. The blocks were mounted for SEM examination and always kept in closed containers to limit any lab contamination. These tissue surfaces were studied with a Hitachi SU6600 field emission SEM with an Oxford EDX with Aztec version 2.0 to 3.3 software, and EDX detector model X-Max 50 SDD. The backscatter mode of the microscope highlighted mineral particles within the tissues. Areas of the tissue at the sectioned block surface were

examined at relatively low magnification 200-500x, and when particles were seen, they were then examined at higher magnification for morphological characteristics and to carry out spectral analysis on each particle found. Electron beam penetration depth under the conditions used was estimated to be 2.5 μm, with an analysis range of 0.5–2.5 μm. Of note, under *in situ* SEM the interior tissue and exterior tissue surfaces were readily distinguishable; this distinction was important for our study. In particular, as subsequent discussion will show, it was important to avoid analyzing surface particulates and instead analyze those inside the tissue. Having a scanned photocopy of the light microscopic slide and the block surface available for reference when performing SEM/EDX helped in navigating the anatomic landmarks, including surface vs. tissue interior location. We subsequently carried out an auxiliary part of this study, in which surface contamination of tissue slides was assessed using two of the cases that had this finding. The surface particles were assessed by *in situ* SEM/EDX to determine the identity (i.e. chemical composition) of the surface contamination.

For this second part of the study, linear regression analyses, with the generation of a coefficient of determination (r) goodness-of-fit value, were done between three statistical pairings: total birefringent particles by light microscopy vs. *in situ* SEM/EDX talc counts, lymph node birefringent particles vs. *in situ* SEM/EDX talc count, and fibroadipose tissue birefringent particles vs. *in situ* SEM/EDX talc counts. Our hypothesis was that the first two pairings would be correlated but the last one would not. The inclusion of multiple specimens from some of the patients meant that the 19 data points (specimens) were not truly independent of each other from the perspective of the population. However, from a statistical point of view, this was justified because, in this phase of our study, the purpose was an evaluation of methods and data related to the samples themselves, and not the population from which the samples were drawn.

Results

Digestion study

Table 1 shows characteristics of the 22 subjects enrolled in the BWH node digestion study, arrayed (least to greatest) by the amount of talc (by digestion) per cm^3 tissue volume. Fourteen (64%) of the women had invasive serous ovarian carcinoma of the ovary, which in one case was mixed with endometrioid carcinoma. Nineteen of the 22 nodes (86%) were external iliac, with 11/19 (58%) from the right side. The age range of the women was 38–73 with a median of 56; 10 (45%) had used talc in their genital area and 16 (73%) had used it as a body powder. There was considerable variation in total talc counts seen after digestion of the nodes. There was also considerable variation in birefringent particle counts in the nodal components, as well as corresponding counts per cm^3 tissue volume (see column totals where pertinent). The number and proportion of nodes with 0, 1, 2, and 3 surface contamination scores were: 4(18%), 7(32%), 7(32%), and 4 (18%).

Of note, cases 4, 9, and 13 had no clinical exposure history, and yet all had high contamination scores (either 2 or 3) and corresponding moderate to high talc counts per cm^3 tissue volume, thus highlighting a role for contamination in their digestion results. In contrast, cases 10 and 18 had clinical **exposure, but** zero contamination scores (i.e. no visible surface contamination); they also had significant talc counts per cm^3 tissue volume, indicating that in the absence of surface contamination, clinical exposure yields significant talc counts using digestion. Case 18 can also be contrasted with cases 19–22, which had the four highest talc counts per cm^3 tissue volume (Table 1), and all of which had high levels of surface contamination.

Table 2 shows Pearson and partial correlations among the various quantitative measurements related to talc and birefringent particles. The degree of surface contamination (0–3 score) as it correlates with other measures of talc and birefringent particles within the node is shown in the right-most column. The surface contamination score was significantly correlated with: the total talc particle count by digestion ($r = 0.43$, $p = 0.05$); with birefringent particle counts by light microscopy in the soft tissue (fibroadipose) component ($r = 0.53$, $p = 0.01$); with total talc per cm^3 tissue volume by SEM/EDX ($r = 0.57$, $p = 0.006$); and with birefringent particle counts in fibroadipose tissue per cm^3 fibroadipose volume ($r = 0.51$, $p = 0.01$). The remainder of correlations and p values in Table 2 represent those for partial correlations

Table 1. Clinical data and talc digestion and light microscopic data among the first patient group (BWH cases).

Case number	Tumor histology	Node*	Component volume (cm^3)			Age	Talc use		Total talc †	Talc/cm^3 of tissue volume	Total birefringence counts††			Birefringence per component volume (particles/ cm^3)			Surface contamination
			Total	Node	Fat		Genital	Body			Total	Node	Fat	Total	Node	Fat	
1	Endometrioid	REI	0.341	0.195 (57%)	0.146 (43%)	60	No	Yes	844	2,475	3750	1250	2500	11,000	6,375	17,250	1
2	Serous invasive	LP	0.334	0.171 (51%)	0.164 (49%)	53	No	Yes	1608	4,800	1250	625	625	3,737	3,661	3,812	1
3	Serous invasive	LEI	0.308	0.119 (39%)	0.188 (61%)	69	No	Yes	2065	6,705	10625	6250	4375	34,552	52,301	23,271	0
4	Serous invasive	LEI	0.407	0.252 (62%)	0.155 (38%)	38	No	No	4290	10,540	4375	1250	3125	11,187	4,960	20,187	2
5	Clear cell	REI	0.332	0.189 (57%)	0.143 (43%)	54	No	Yes	3965	11,942	15000	12500	2500	45,146	66,286	17,406	0
6	Serous invasive	REI	0.232	0.169 (73%)	0.063 (27%)	50	Yes	No	3378	14,500	1250	625	625	5,387	3,687	9,937	1
7	Endometrioid	REI	0.557	0.392 (70%)	0.165 (30%)	46	No	No	8920	16,000	4375	1250	3125	7,912	3,187	18,937	1
8	Serous invasive	LEI	0.107	0.039 (36%)	0.069 (64%)	49	Yes	Yes	2533	23,562	1250	0	1250	11,687	0	18,375	1
9	Endometrioid	REI	0.533	0.089 (17%)	0.444 (83%)	57	No	No	19,094	35,823	15000	3125	11875	28,103	35,014	26,715	2
10	Granulosa cell	REI	0.237	0.206 (87%)	0.030 (13%)	49	Yes	Yes	20,267	85,600	4,375	3,125	1,250	18,500	15,125	41,375	0
11	Serous invasive	REI	0.107	0.092 (86%)	0.015 (14%)	51	No	No	10,390	97,100	5,000	625	4,375	46,750	6,812	291,687	2
12	Serous invasive	RP	0.026	0.021 (79%)	0.006 (21%)	51	Yes	Yes	2,834	107,300	10,625	5,625	5,000	402,437	269,125	908,750	2
13	Serous invasive	LEI	0.147	0.022 (15%)	0.125 (85%)	68	No	No	16,057	115,030	16,875	1,250	15,625	114,812	56,562	125,125	3
14	Serous invasive	REI	0.219	0.145 (66%)	0.074 (34%)	73	Yes	Yes	30,330	138,500	8,125	1,875	6,250	37,062	12,937	84,437	2
15	Endometrioid	REI	0.506	0.083 (16%)	0.423 (84%)	58	Yes	Yes	73,267	144,800	26,875	12,500	14,375	53,125	151,500	33,937	2
16	Serous borderline	REI	0.147	0.055 (37%)	0.092 (63%)	60	No	Yes	21,409	145,600	11,875	2,500	9,375	80,812	45,437	101,875	1
17	Serous invasive	LEI	0.174	0.123 (71%)	0.051 (29%)	62	Yes	Yes	33,778	194,100	30,625	28,125	2,500	176,000	228,687	49,437	1
18	Serous invasive	LEI	0.323	0.203 (63%)	0.121 (37%)	53	Yes	Yes	67,557	208,200	>125,000	>125,000	625	>387,000	>616,365	3,000	0
19	Serous invasive	LEI	0.052	0.017 (33%)	0.035 (67%)	69	No	Yes	12,661	242,100	11,250	1,250	10,000	215,000	71,875	285,625	3
20	Serous invasive	LEI	0.286	0.185 (65%)	0.101 (35%)	66	Yes	Yes	92,891	325,200	4,375	3,125	1,250	15,312	16,875	12,437	2
21	Endometrioid	REI	0.056	0.039 (70%)	0.017 (30%)	51	No	Yes	85,041	1,518,589	13,750	1,250	12,500	246,875	32,051	735,294	3
22	Serous/ endometrioid	RPA	0.424	0.284 (67%)	0.139 (33%)	69	Yes	Yes	797,171	1,881,500	>62,500	>62,500	1,250	>147,500	>220,062	9,000	3
Median			0.262	0.134	0.111	56			14,359	102,200	10,625	2,188	3,125	41,104	33,533	24,993	

*Location of Node: LEI = Left external iliac; REI = Right external iliac; RPA = Right paraaortic; LP = Left pelvic; RP = Right pelvic

†Total number of talc particles by digestion (calculated)

††Total birefringence counts = particles in field x 625 (see Materials and Methods)

Node refers to lymph node parenchyma areas as measured by Image J software and studied by light microscopy (see Materials and Methods).

Fat refers to fibroadipose soft tissue areas as measured by Image J software and studied by light microscopy

Table 2. Correlations between surface contamination, talc, and age (r and p values).

Variable*	Surface contamination r (p)	Total talc by digestion r (p)	Total birefringent partide counts			Talc/total volume r (p)	Birefringent particle counts per cm^3 volume		
			Total r (p)	Node r (p)	Fat r (p)		Total r (p)	Node r (p)	Fat r (p)
Total talc by digestion	0.43 (0.05)								
Total birefringent particle counts	0.15 (0.51)	0.67 (0.001)							
Total birefringent particle counts, node	0.07 (0.77)	0.59 (0.005)	0.81 (<.0001)						
Total birefringent particle counts, fat	0.53 (0.01)	0.13 (0.58)	0.25 (0.26)	0.07 (0.76)					
Talc/cm^3 volume	0.57 (0.006)	0.87 (<.0001)	0.63 (0.002)	0.47 (0.03)	0.06 (0.78)				
Birefringent particles per cm^3 total volume	0.33 (0.13)	0.42 (0.06)	0.82 (<.0001)	0.56 (0.008)	0.3 (0.19)	0.68 (0.0007)			
Birefringence per cm^3 node volume	0.07 (0.77)	0.51 (0.02)	0.90 (<.0001)	0.88 (<.0001)	0.18 (0.45)	0.64 (0.003)	0.87 (<.0001)		
Birefringence per cm^3 fat volume	0.51 (0.01)	0.24 (0.30)	0.003 (0.99)	0.1 (0.68)	0.61 (0.003)	0.16 (0.48)	0.45 (0.04)	0.13 (0.58)	
Age	0.28 (0.20)	0.26 (0.26)	0.35 (0.12)	0.32 (0.15)	0.16 (0.49)	0.22 (0.33)	0.26 (0.25)	0.36 (0.12)	0.07 (0.75)

Node = lymph node tissue
Fat = fibroadipose tissue

adjusted for the level of surface contamination. Not unexpectedly, total counts always strongly correlated with counts per cm^3 of relevant tissues: e.g. total talc with total talc per cm^3 tissue volume (r = 0.87, p = 0.001); total birefringent particle counts in lymph node tissue with birefringent counts per cm^3 lymph node tissue (r = 0.88, p = 0.0001); and birefringent particle counts in fibroadipose tissue with birefringence counts per cm^3 fibroadipose volume (r = 0.61, p = 0.003). Talc counts per cm^3 tissue volume correlated with: birefringent particles per cm^3 tissue volume (r = 0.68, p = 0.007), and lymph node birefringent particles per cm^3 lymph node tissue (r = 0.64, p = 0.003), but not with fibroadipose birefringent particles per cm^3 fibroadipose tissue. Total birefringent particles per cm^3 tissue volume correlated best with lymph node birefringent particles per cm^3 lymph node tissue (r = 0.89, p = 0.001). Birefringent particle counts per cm^3 lymph node tissue were not correlated with fibroadipose birefringent particle counts per cm^3 fibroadipose volume. Age was not significantly correlated with any measure of nodal contamination.

Figure 2 and Table 3 illustrates the potential effect of surface contamination on the interpretation of the relationship between total talc (by digestion) per cm^3 tissue volume. Figure 2 illustrates that for any level of surface contamination, those who used talc in the genital area had a higher amount of talc than those who had not used talc genitally. Table 3 quantifies

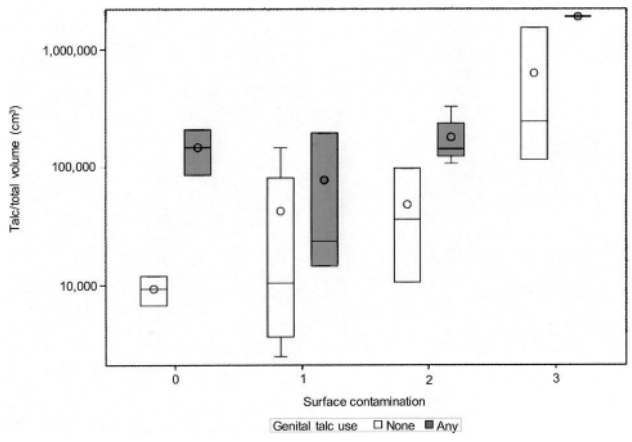

Figure 2. Talc/total volume for genital talc users and non-users by surface contamination This figure shows surface contamination scores (x axis) plotted against talc per tissue volume (y-axis, logarithmic scale), showing that for any level of surface contamination, those who used talc in the genital area had a higher amount of talc than those who had not used talc genitally.

Table 3. Geometric mean talc/total volume by genital talc use.

Talc/total volume	No genital talc use (n = 12) Geometric mean (95% CI)	Any genital talc use (n = 10) Geometric mean (95% CI)	p-value
Crude	35,049 (13,637, 90,079)	131,584 (46,787, 370,070)	0.08
Adjusted for surface contamination	29,926 (15,546, 57,605)	159,056 (77,491, 326,475)	0.004

this effect more precisely and indicates that, overall, the genital talc user had higher talc counts per volume of tissue than those who had not used talc, but the association was of borderline significance. After adjustment for level of surface contamination, the association became significant (p = 0.004) with the level of talc in nodal tissue at least five times higher in those who used talc genitally compared to those who had not.

Figure 3 shows correlative polarizing light microscopy, SEM, and EDX from case 18 in the digestate study (Table 1). Going clockwise from upper left, panel **A** shows polarized light microscopy (H&E, 200x), showing numerous birefringent particles (general size range 1 to 5 μm) within the macrophages of a left external iliac lymph node. This case was near the upper end of the range of particle abundances we observed. Panel **B** shows examples of two particles (labeled 1103 and 1104), identified by SEM on the digestate filter, each <5 μm diameter. Panel **C** shows the spectrum for particle 1103, with an Mg-Si atomic weight ratio of 0.6495, characteristic of talc. The other particle in **B**, 1104, had an Mg-Si ratio within 5% of the theoretical talc value (0.649).

In situ *SEM study*

Table 4 shows data for the second part of the study (19 lymph node specimens from 10 patients). The left-most two columns (case number and block letter) are

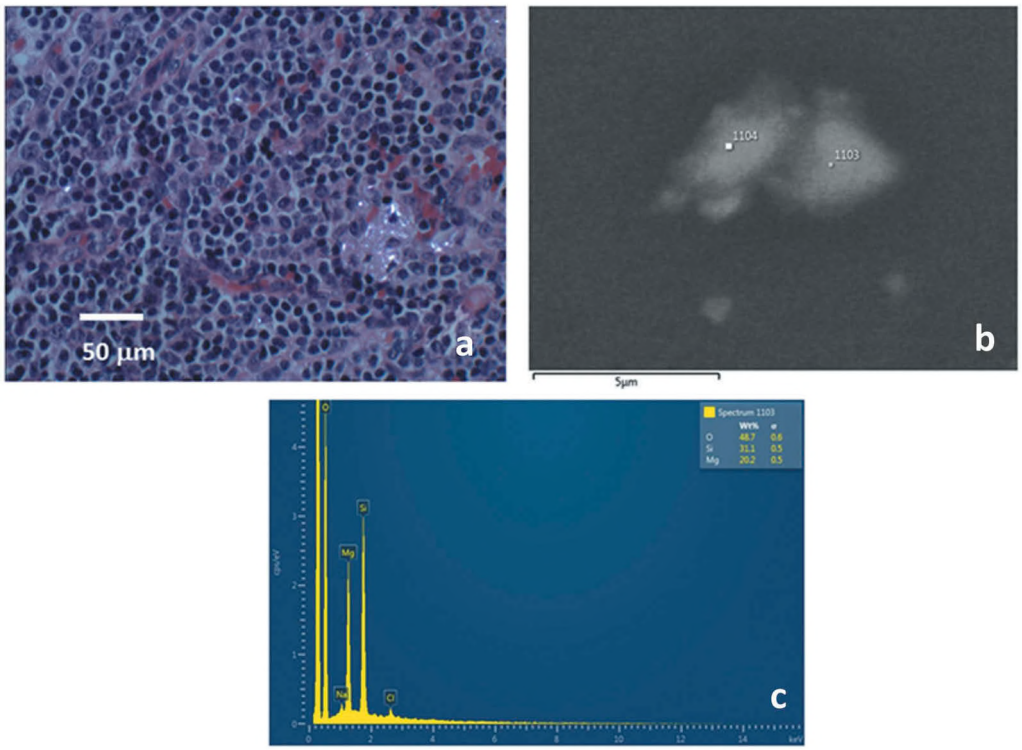

Figure 3. Correlative polarizing light microscopy, SEM, and EDX from case 18 in the digestate study (Table 1). Clockwise from upper left: **a**, Polarizing light microscopy, H&E, 200x, showing numerous birefringent particles (general size range 1 to 5 μm) within the macrophages of a left eternal iliac lymph node. **b**, Two particles (labeled 1103 and 1104), identified by SEM on the digestate filter, each <5 μm diameter. **c**, Spectrum for particle 1103, The Mg-Si atomic weight ratio is 0.6495, characteristic of talc. The other particle in **b**, 1104, had an Mg-Si atomic weight ratio within 5% of the theoretical talc value (0.649).

Table 4. Correlation between light microscopic birefringent particulates and in situ SEM analysis for talc particles.

Case number	Slide letter	Birefringent particles in lymph node tissue (total per slide)	Birefringent particles in surrounding fibroadipose tissue (total per slide)	Total birefringent particles in slide (columns C + D)	Number of talc particles in the block by in situ SEM
1	A	3	5	8	0
	B	55	7	62	5
2	A	5	2	7	9
3	A	2	0	2	0
	B	0	0	0	0
4	A	19	9	28	31
	B	3	1	4	5
5	A	>500	3	>500	65
6	A	6	4	10	0
	B	8	3	11	0
	C	16	4	20	0
7	A	7	3	10	1
	B	1	0	1	0
8	A	>100	3	>100	18
	B	>200	2	>200	43
	C	>100	5	>100	35
	D	>100	7	>100	24
9	A	8	6	14	1
10	A	15	>50	>50	12

In this part of the study, 19 pelvic lymph node slides on 10 ovarian carcinoma patients (with each patient having from one to four node specimens), showed the relationship of the numbers of birefringent particles (by light microscopy) within histological sections (separately categorized in lymph node and fibroadipose tissue components), and talc particles found by SEM/EDX at deeper levels in the tissue blocks corresponding to those sections (right-hand column). In case 9C, the vast majority of the birefringent particles were localized in only one of several lymph nodes visible in the slide. Note that cases with very numerous particle counts by light microscopy are designated simply as greater than a certain threshold.

fully de-identified and serve for identification purposes within the table only. The table shows the relationship of the numbers of birefringent particles by light microscopy within histological sections (separately categorized in lymph node and fibroadipose tissue components), and talc particles found by SEM/EDX on the block surface (following the preparation procedure) corresponding to those sections (right-most column). Consistent with our hypotheses, strong correlations using Spearman correlations were indeed evident between a) lymph node counts by light microscopy and the SEM total talc count ($r = 0.80$, $p < 0.0001$); and b) total particle counts by light microscopy and the SEM total talc count ($r = 0.79$, $p < 0.0001$). Fibroadipose tissue counts by light microscopy did not correlate with SEM total talc counts ($r = 0.32$, $p =$ not significant). In controlling for correlated observations from the same patient, Spearman correlations using one record per case were done for the six patients where more than one lymph node specimen was included in the study (among these patients, the specimen with the highest SEM talc count was the one selected). With this adjustment, strong correlations were still observed using Spearman correlations as evident between a) lymph node counts by light microscopy and the SEM total talc count ($r = 0.69$, $p < 0.03$); and b) total particle counts by light microscopy and the SEM total talc count ($r = 0.74$, $p < 0.01$), Fibroadipose tissue counts by light microscopy did not correlate with SEM total talc counts ($r = 0.16$, $p =$ not significant).

Figure 4 shows correlative polarizing light microscopy, *in situ* SEM, and EDX on case 9C from Table 4. Going clockwise from lower left, panel **A** shows numerous birefringent particles under polarized light microscopy (H&E, 400x) within the macrophages of a left external iliac lymph node. Panel **B** shows low-power backscattered electron imaging under SEM with several positive particles. Panel **C** shows an enlarged (cropped) view of the lower right-hand part of panel B. Three particles are labeled – 44, 45, and 46. Panel **D** shows the spectrum for particle 45, which showed an Mg-Si ratio of 0.643. Particle 44 was also within the 5% of the theoretical value of 0.649 and so was considered talc as well. Particle 46 had an Mg-Si ratio of 0.610, which falls just outside the 0.649 ± 5% range for talc, and so it was considered a nonspecific magnesium silicate.

A review of the non-talc particles found by *in situ* SEM in the 10 patients in Table 4 showed an aggregated total of 310, which based on their chemical composition would be regarded as likely birefringent. Of these, the most common were magnesium silicates outside the 5% theoretical range of the Mg-Si atomic weight spectral ratio for talc (113 total particles or 36%), aluminum silicates with or without magnesium (91 total particles or 29%), and calcium without phosphate (41, or 13%), with others accounting for the remaining 22%. Non-fibrous, non-talc silicates are known to have a longer dissolution time than talc in physiologic conditions; the dissolution time for talc is approximately 8 years for a 1 μm particle.[19] Thus, the component of non-talc silicates in pelvic tissues could proportionally rise over sufficient

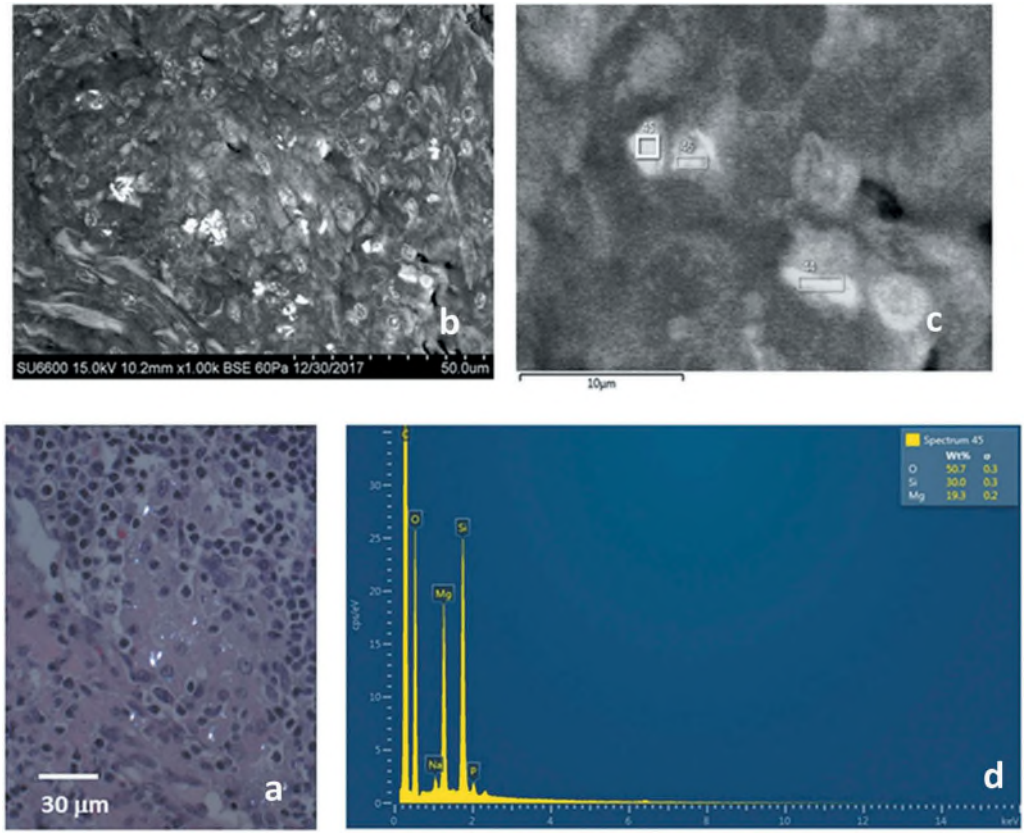

Figure 4. Correlative polarizing light microscopy, in situ SEM, and EDX on case 8C from Table 4. Clockwise from lower left: **a**, Numerous birefringent particles under polarized light microscopy (H&E, 400x) within the macrophages of a left external iliac lymph node. **b**, Low-power backscattered electron imaging under SEM with several positive particles. **c**, Enlarged (cropped) lower right-hand portion of **b**. Three particles are labeled – 44, 45, and 46. **d**, Spectrum for particle 45, which showed an Mg-Si atomic weight ratio of 0.643. Particle 44 was also within the 5% of the theoretical value of 0.649 and so can be considered talc as well. Particle 46 had an Mg-Si atomic weight ratio of 0.610, which falls just outside the 5% range for talc and so can be considered a nonspecific magnesium silicate.

elapsed time (years), even if the original exposure to talc was heavy.

To provide final evidence for our hypothesis that talc is an important part of specimen surface contamination, two authors (SM and JG) re-reviewed the 19 slides from the second part of the study (*in situ* SEM). The goal was to find cases in this group with surface contamination. We did not find any with a score of 3, but two cases (1B and 7A from Table 4) were chosen that, respectively, had contamination scores of 2 and 1 (with 100% agreement by pathologists SM and JG), and substantial amounts of evaluable surface area. On polarizing light microscopy, these cases showed a mixture of larger paper debris fragments and smaller (1–10 µm) birefringent particulates along the surface similar to those previously seen for many Table 1 cases., Respectively, for 1B and 7A, 13 and 5 small birefringent particulates were found by thorough examination of their surfaces in addition to larger paper debris. SEM of the tissue surface for block 1B (35 mm^2 analysis area) showed a total of 5 talc particles, and for 7A showed 1 talc particle (50 mm^2 analysis area). Given the 2.5 µm effective section thickness (electron beam analysis depth) and these relatively small surface areas, these SEM talc particle counts are significant, and are consistent with the light microscopic review. Thus, this portion of the study directly showed that surface contamination particles were talc, whereas previously, this had only been strongly implied by the results in Table 1. (See supplementary figure S1). In addition to the talc particles, 44 other exogenous particles were found across tissue surfaces of these two cases by SEM/EDX: 27 external mineral (mainly Si in combination with Mg and/or Al), 6 non-talc Mg-Si minerals, and 11 external metal.

Discussion

The accurate identification of talc in pelvic tissues is important because it documents exposure by demonstrating the presence of talc in these tissues and provides evidence in support of the role of talc in the epidemiological association with ovarian cancer in case-control studies.[9–13,15] The overall relative risk across the various positive studies is around 1.3, and where tumor histology data have been available for review, several common subtypes (serous carcinoma, endometrioid carcinoma, and serous borderline tumors) are most frequently involved in the association.[11,13]

Talc, when applied to the perineum, is believed to migrate to the upper genital tract, passing through the open tract to the fallopian tubes and eventually reaching the ovaries.[11,16] Talc may also gain access to the lymphatic system as a means of reaching pelvic organs and lymph nodes,[20,21], similar to the route to the pulmonary nodes of talc miners.[22] Lymph nodes of the pelvic region include several anatomic sub-classifications (inguinal, iliac, and paraaortic), with the common theme that they may receive lymphatic efferents from pelvic organs such as the ovaries and perineum and/or secondarily from other lymph nodes in the area. Ovarian carcinoma, especially serous, tends to metastasize early (when just one or two nodes are involved) to paraaortic nodes.[23] Full discussions of the lymphatic drainage/anatomy of the pelvic region are available in the literature.[20,21] Lymph nodes are often sampled during gynecologic surgery for tumor staging and assessment for metastatic disease. However, additional examination of these nodes for talc, especially in settings where genital exposure is known to have occurred, would add insight as to the ability of talc to migrate and lodge within pelvic tissues.

This study supports earlier observations that talc particles, from perineal exposure, can and do migrate to pelvic lymph nodes. Material with the microscopic and spectral features of talc was clearly demonstrated within the lymph node parenchyma in most of our cases, as scattered birefringent particles in the general size range 1–10 μm. Sometimes the material was visible within nodal macrophages, lending strong credence to a lymphatic migration route. Similar particles were also found in the fibroadipose tissue adjacent to lymph nodes, where they may have arrived via the lymphatic system, but more likely resulted from visibly present surface contamination pushed into the underlying fibroadipose tissue.

Our study took the additional critical step of comparing the light microscopic data to SEM digestion data, thereby going beyond the earlier study by Heller et al.[17] in scope, in addition to examining lymph nodes rather than ovaries. Like that earlier paper, we found high talc particle burdens in some digested samples. But because these correlated with contamination scores, we believe that the digestion counts are not fully reflective of clinically relevant talc exposure or its migration in the tissues. Instead, they are influenced by contamination, such as talc introduced by non-surgical gloves used for handling tissue and in the general lab environment during tissue collection and processing in the pathology laboratory. Thus, tissue digestion should not be regarded as a reliable quantification method for talc or contaminants of talc, especially where the collection and processing steps have not been rigidly controlled from the start. The correlation of contamination scores with counts of birefringent particles in fibroadipose tissue suggests that particles adherent to the surface (through contamination) may be pushed into the soft fibroadipose tissue, since it is typically the most peripheral type of tissue, with the nodal tissue usually deeper and encapsulated with a fibrous tissue capsule. The highly variable talc burdens found by digestive analysis and SEM, spanning approximately three orders of magnitude, are consistent with contamination influence, since the latter would be expected to vary considerably between procurement environments. However, this could also be observed in the range of burdens seen in a clinically exposed population with appropriate lab procedures/controls (Table 4).

Even though contamination played a role in total tissue counts, it was still the case that high talc burdens in the lymph nodes, when present, contributed to the SEM digestate results, hence producing the observed correlation between the two. Thus, it is likely that both contamination and clinically significant lymph node talc are reflected in the SEM digestate data. The main

problem in using digestion is that it likely raises the baseline for all patients and groups, thus potentially obscuring clinically significant differences, which would otherwise be observed if contamination were eliminated (as previously mentioned, Table 3 illustrates a robust demonstration of this effect).

By showing strong correlations between particle counts (polarized light microscopy) and *in situ* SEM analysis, the second part of our study demonstrated that the latter alternative is a better method of talc assessment than digestion, because the anatomic landmarks are preserved and surface contamination is not incorporated into the general talc count, as it is with tissue digestion. In combination with other parts of our study, this aspect also showed that the birefringent material in the lymph node tissue, is the clinically significant component related to talc exposure. Surface contamination can still be present, and our demonstration of talc on the surfaces of cases 1B and 7A by *in situ* SEM lent support to the conclusions from the first (digestion) part of the study.

A major strength of our study was the correlative light microscopic and SEM/EDX data for each case, with examination of anatomic locations in the former. This provided a key perspective in the evaluation of the talc burden data that a digestive study alone would not have given. In fact, this study demonstrates the broader principle that correlative histologic review is important in many areas of pathology – especially where digestion procedures are performed, and where the study of anatomic landmarks are needed to complement data from the latter. This is because the tissue is compartmentalized histologically, with different functions and significance for each component, a fact not always recognized by those who digest tissue routinely and use the resulting product completely in analyses such as Western blotting or mutational assays.[24]

Unfortunately, as part of our study, we were not able to also do *in situ* SEM/EDX on the intact tissues used for digestion in the first group of cases (22 patients). However, by showing that birefringent particles within lymph nodes were strongly correlated with the demonstration of talc inside the nodes by *in situ* SEM/EDX, the second part of our study filled that role, and thus 1) material in lymph nodes is likely reflective of the clinical exposure, 2) in this clinical setting and given our results, a substantial proportion of this birefringent material is likely to be talc, 3) surface contamination is common, and so with *in situ* SEM, it is important to discern the anatomic landmarks, and avoid analyzing surface particulates (as shown by our direct demonstration of talc on the surfaces of cases 1B and 7A in our auxiliary study to the cases in Table 4).

In addition to talc, much other commonly found birefringent material, such as that described in the Results section for the SEM analysis, is likely nonspecific particulate material which finds its way into the perineum through general living and hygiene practices. Another important point is that seeing particles by *in situ* microscopy, both light and SEM, requires a relatively large amount of material distributed within the tissues in order to find it. As a demonstration of this principle, Roggli and Pratt[25] showed that finding one asbestos body in a tissue section was indicative of at least 100 fibers per gram of tissue. The calculations we used to estimate particles/cm^3 of tissue volume (Table 1), starting with a count of birefringent particles in tissue sections, illustrate a similar principle.

In the long-studied and debated association between talc exposure and ovarian cancer, our study provides additional evidence that talc may enter pelvic tissues and ultimately be detected and measured in regional lymph nodes, and this relationship became especially strong when clinical use data was considered and surface contamination was corrected for statistically. This adds perspective to the known migratory capabilities and overall biological role/impact of talc. For some of the more heavily exposed cases in the second part of the study, we noticed that the large majority of birefringent material was localized in a single node, among several present on a given slide. This suggested that pelvic drainage/migration pathways for talc may be very specific, and focused on one or relatively few nodes as an endpoint – perhaps consistent with the concept of sentinel nodes in oncologic surgery.[26]

Our findings also suggest that in patients with ovarian cancer, clinicians may want to make broader inquiries into the past and present use

of talc by their patients. Similarly, pathologists may wish to pay greater attention to sampled regional lymph nodes. In addition to the usual study of these nodes for metastases, they may wish to examine macrophages more closely for exogenous particles including by polarized light. A positive finding may trigger clinical inquiries about exposure where it was not previously suspected. Our findings yield important insights as to the ability of talc to migrate to nodes, and under what conditions its identification in nodes and tissues is clinically meaningful and when not.

In conclusion, talc contamination of the surface of surgical pathology specimens is common. Exposure (such as perineal application), whether known clinically or not, often results in significant deposition of talc in the tissues. Correlative light microscopy is needed to assess the possibility of lab contamination, and to determine if talc is truly present in clinically meaningful locations in lymph nodes or other tissues.

Declaration of Interest Statement

The authors declare the following competing financial interest(s): JJG, DC and WW have served as consultants and provided expert testimony in talc and other environmental litigation. SM, YF, RS, MK, and LS report no conflicts of interest.

Funding

Funding for this research was provided through the Harvard NIEHS Center (ES-000002) Particles Research Core and Integrated Facilities Core, NIH grants R01CA054419 and P50CA105009, the authors, and through John J. Godleski, MD PLLC.

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Exhibit I

Original Article

Reproductive Sciences
1-10

DOI: 10.1177/1933719119831773
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Molecular Basis Supporting the Association of Talcum Powder Use With Increased Risk of Ovarian Cancer

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Abstract

Genital use of talcum powder and its associated risk of ovarian cancer is an important controversial topic. Epithelial ovarian cancer (EOC) cells are known to manifest a persistent prooxidant state. Here we demonstrated that talc induces significant changes in key redox enzymes and enhances the prooxidant state in normal and EOC cells. Using real-time reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay, levels of CA-125, caspase-3, nitrate/nitrite, and selected key redox enzymes, including myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GSR), were determined. TaqMan genotype analysis utilizing the QuantStudio 12K Flex was used to assess single-nucleotide polymorphisms in genes corresponding to target enzymes. Cell proliferation was determined by MTT proliferation assay. In all talc-treated cells, there was a significant dose-dependent increase in prooxidant iNOS, nitrate/nitrite, and MPO with a concomitant decrease in antioxidants CAT, SOD, GSR, and GPX ($P < .05$). Remarkably, talc exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. Talc exposure also resulted in a significant increase in inflammation as determined by increased tumor marker CA-125 ($P < .05$). More importantly, talc exposure significantly induced cell proliferation and decreased apoptosis in cancer cells and to a greater degree in normal cells ($P < .05$). These findings are the first to confirm the cellular effect of talc and provide a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.

Keywords

talc, epithelial ovarian cancer, oxidative stress, single-nucleotide polymorphism, cell proliferation

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and ranks fifth in cancer deaths among women diagnosed with cancer.[1] Epithelial ovarian cancer (EOC) has long been considered a heterogeneous disease with respect to histopathology, molecular biology, and clinical outcome.[1,2] Although surgical techniques and treatments have advanced over the years, the prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage.[2] This is largely due to the lack of early warning symptoms and screening methods and the development of chemoresistance.[1,2] Moreover, ovarian cancer is known to be associated with germline mutations in the *BRCA1* or *BRCA2* genes, but with a rate of only 20 % to 40%, suggesting the presence of other unknown mutations in other predisposition genes.[3] Additional genetic variations including single-nucleotide polymorphisms (SNPs) have been hypothesized to act as low to moderate penetrant alleles that contribute to ovarian cancer risk.[3,4]

The pathophysiology of EOC is not fully understood but has been strongly associated with inflammation and the resultant oxidative stress.[5] We have previously characterized EOC cells to manifest a persistent prooxidant state as evident by the upregulation of key oxidants and downregulation of key antioxidants, which is further enhanced in chemoresistant EOC cells.[6] The expression of key prooxidant/inflammatory enzymes such as inducible nitric oxide synthase (iNOS), nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, and myeloperoxidase (MPO), as well as an increase in nitric oxide (NO) levels, was increased in EOC tissues and cells.[6] Additionally, we have shown that EOC cells manifest lower apoptosis, which

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was markedly induced by inhibiting iNOS, indicating a strong link between apoptosis and NO/iNOS pathways in these cells.[6]

The cellular redox balance is maintained by key antioxidants including catalase (CAT), superoxide dismutase (SOD), or by glutathione peroxidase (GPX) coupled with glutathione reductase (GSR).[5] Other important scavengers include thioredoxin coupled with thioredoxin reductase, and glutaredoxin, which utilizes glutathione (GSH) as a substrate.[7] We have previously reported that a genotype switch in key antioxidants is a potential mechanism leading to the acquisition of chemoresistance in EOC cells.[7] We have studied the effects of genetic polymorphisms in key redox genes on the acquisition of the oncogenic phenotype in EOC cells, including genes that control the levels of cellular reactive oxygen species and oxidative damage and SNPs for genes involved in carcinogen metabolism (detoxification and/or activation), antioxidants, and DNA repair pathways.[4,6] Several function-altering SNPs have been identified in key antioxidants, including CAT, GPX, GSR, and SOD.[4]

Several studies have suggested the possible association between genital use of talcum powder and risk of EOC.[7-12] Association between the use of cosmetic talc in genital hygiene and ovarian cancer was first described in 1982 by Cramer et al, and many subsequent studies supported this finding.[7-12] Talc and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature.[7-12] Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response.[7] The objective of this study was to determine the effects of talcum powder on the expression of key redox enzymes, CA-125 levels, and cell proliferation and apoptosis in normal and EOC cells.

Material and Methods

Cell Lines

Ovarian cancer cells SKOV-3 (ATCC), A2780 (Sigma Aldrich, St Louis, Missouri), and TOV112D (a kind gift from Gen Sheng Wu at Wayne State University, Detroit, Michigan) and normal cells human macrophages (EL-1; ATCC, Manassas, Virginia), human primary normal ovarian epithelial cells (Cell Biologics, Chicago, Illinois), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc, Carlsbad, California), and immortalized human fallopian tube secretory epithelial cells (FT33; Applied Biological Materials, Richmond, British Columbia, Canada) were used. All cells were grown in media and conditions following manufacturer's protocol. EL-1 cells were grown in IMDM media (ATCC) supplemented with 0.1 mM hypoxanthine and 0.1 mM thymidine solution (H-T, ATCC) and 0.05 mM β-mercaptoethanol. SKOV-3 EOC cells were grown in HyClone McCoy's 5A medium (Fisher Scientific, Waltham, Massachusetts), A2780 EOC cells were grown in HyClone RPMI-1640 (Fisher Scientific), and both TOV112D EOC cells were grown in MCDB105 (Cell Applications, San Diego, California) and Medium 199 (Fisher Scientific; 1:1). All media were supplemented with fetal bovine serum (Innovative Research, Novi, Michigan) and penicillin/streptomycin (Fisher Scientific), per their manufacturer specifications. Human primary normal ovarian epithelial cells were grown in complete human epithelial cell medium (Cell Biologics).

Treatment of Cells

Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) was dissolved in dimethyl sulfoxide (DMSO; Sigma Aldrich) at a concentration of 500 mg in 10 mL and was filtered with a 0.2 μm syringe filter (Corning). Sterile DMSO was used as a control for all treatments. Cells were seeded in 100-mm cell culture dishes (3 10^6) and were treated 24 hours later with 5, 20, or 100 μg/mL of talc for 72 hours. Cell pellets were collected for RNA, DNA, and protein extraction. Cell culture media were collected for CA-125 analysis by enzyme-linked immunosorbent assay (ELISA).

Real-Time Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from all cells using the RNeasy mini kit (Qiagen, Valencia, California). Measurement of the amount of RNA in each sample was performed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). A 20 μL complementary DNA reaction volume containing 0.5 μg RNA was prepared using the SuperScript VILO Master Mix Kit (Life Technologies, Carlsbad, California). Optimal oligonucleotide primer pairs were selected for each target using Beacon designer (Premier Biosoft, Inc; Table 1). Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using the EXPRESS SYBR GreenER qPCR supermix kit (Life Technologies) and the Cepheid 1.2f detection system (Sunnyvale, CA) previously described.[6] Standards with known concentrations and lengths were designed specifically for β-*actin* (79 bp), *CAT* (105 bp), *NOS2* (89 bp), *GSR* (103 bp), *GPX1* (100 bp), *MPO* (79 bp), and *SOD3* (84 bp), allowing for construction of a standard curve using a 10-fold dilution series.[6] All samples were normalized to β-*actin*. A final melting curve analysis was performed to demonstrate specificity of the PCR product.

Protein Detection

Cell pellets were lysed utilizing cell lysis buffer (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin) containing a cocktail of protease inhibitors. Samples were centrifuged at 13 000 rpm for 10 minutes at 4 C. Total protein concentration of cell lysates from control and talc-treated cells was measured with the Pierce BCA protein assay kit (Thermo Scientific, Rockford, Illinois).

Table 1. Real-Time RT-PCR Oligionucleotide Primers.

Accession Number	Gene	Sense (5′-3′)	Antisense (3′-5′)	Amplicon (bp)	Annealing Time (seconds) and Temperature (C)
NM_001101	β-*actin*	ATGACTTAGTTGCGTTACAC	AATAAAGCCATGCCAATCTC	79	10, 64
NM_001752	*CAT*	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	10, 63
NM_003102	*SOD3*	GTGTTCCTGCCTGCTCCT	TCCGCCGAGTCAGAGTTG	84	60, 64
NM_000637	*GSR*	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	116	10, 63
NM_000581	*GPX1*	GGACTACACCCAGATGAAC	GAGCCCTTGCGAGGTGTAG	91	10, 66
NM_000625	*NOS2*	GAGGACCACATCTACCAAGGAGGAG	CCAGGCAGGCGGAATAGG	89	30, 59
NM_000250	*MPO*	CACTTGTATCCTCTGGTTCTTCAT	TCTATATGCTTCTCACGCCTAGTA	79	60, 63

Abbreviation: RT-PCR, reverse transcription polymerase chain reaction.

Detection of Protein/Activity by ELISA

The following ELISA kits were used (Cayman Chemical, Ann Arbor, Michigan): CAT, SOD, GSR, GPX, and MPO. Nitrite (NO_2^-)/nitrate (NO_3^-) were determined spectrophotometrically by Griess assay as previously reported.[6] CA-125 protein levels were measured in cell media by ELISA (Ray Biotech, Norcross, Georgia).

TaqMan SNP Genotyping Assay

DNA was isolated utilizing the EZ1 DNA tissue kit (Qiagen) for EOC cells. The TaqMan SNP genotyping assay set (Applied Biosystems, Carlsbad, California; NCBI dbSNP genome build 37, MAF source 1000 genomes) was used to genotype the SNPs (Table 1). The Applied Genomics Technology Center (AGTC, Wayne State University) performed these assays. Analysis was done utilizing the QuantStudio 12 K Flex real-time PCR system (Applied Biosystems).

Cell Proliferation and Apoptosis

Cell proliferation was assessed with the TACS MTT cell proliferation assay (Trevigen, Gaithersburg, Maryland) after treatment with talc (100 μg/mL) for 24 hours. The Caspase-3 Colorimetric Activity Assay Kit (Chemicon, Temecula, California) was used to determine levels of caspase-3 activity after treatment of normal and EOC cells with various doses of talc as previously described.[6] Equal concentrations of cell lysate were used. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate DEVD-pNA. The free pNA can be quantified using a spectrophotometer or a microtiter plate reader at 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with its control allows determination of the percentage increase in caspase-3 activity.

Statistical Analysis

Normality was examined using the Kolmogorov-Smirnov test and by visual inspection of quantile–quantile plots. Because most of the data were not normally distributed, differences in distributions were examined using the Kruskal-Wallis test. Generalized linear models were fit to examine pairwise differences in estimated least squares mean expression values by exposure to 0, 5, 20, or 100 μg/mL of talc. We used the Tukey-Kramer adjustment for multiple comparisons, and the regression models were fit using log2 transformed analyte expression values after adding a numeric constant "1" to meet model assumptions while avoiding negative transformed values. P values below .05 are statistically significant.

Results

Talc Treatment Decreased the Expression of Antioxidant Enzymes SOD and CAT in Normal and EOC Cells

Real-time RT-PCR and ELISA assays were utilized to determine the CAT and SOD messenger RNA (mRNA) and protein levels in cells before and after 72 hours talc treatment, respectively (Figure 1). The CAT (Figure 1A and C) and SOD (Figure 1B and D) mRNA and protein levels were significantly decreased in a dose-dependent manner in talc-treated cells compared to controls ($P < .05$).

Talc Treatment Increased the Expression of Prooxidants iNOS, NO_2^-/NO_3^-, and MPO in Normal and EOC Cells

Real-time RT-PCR and NO_2^-/NO_3^- assays were utilized to determine the iNOS mRNA and NO levels in cells before and after 72 hours talc treatment, respectively (Figure 2). The iNOS mRNA and NO levels were significantly increased in a dose-dependent manner in talc-treated cells as compared to their controls (Figure 2A and C, $P < .05$). As expected, there was no detectable MPO in normal ovarian and fallopian tube cells, and thus, talc treatment did not have any effect. However, MPO mRNA and protein levels were significantly increased in a dose-dependent manner in talc-treated ovarian cancer cells and macrophages compared to controls (Figure 2B and D, $P < .05$).

Talc Treatment Decreased the Expression of Antioxidant Enzymes, GPX and GSR, in Normal and EOC Cells

Real-time RT-PCR and ELISA assays were utilized to determine the GPX and GSR mRNA and protein levels in cells before and

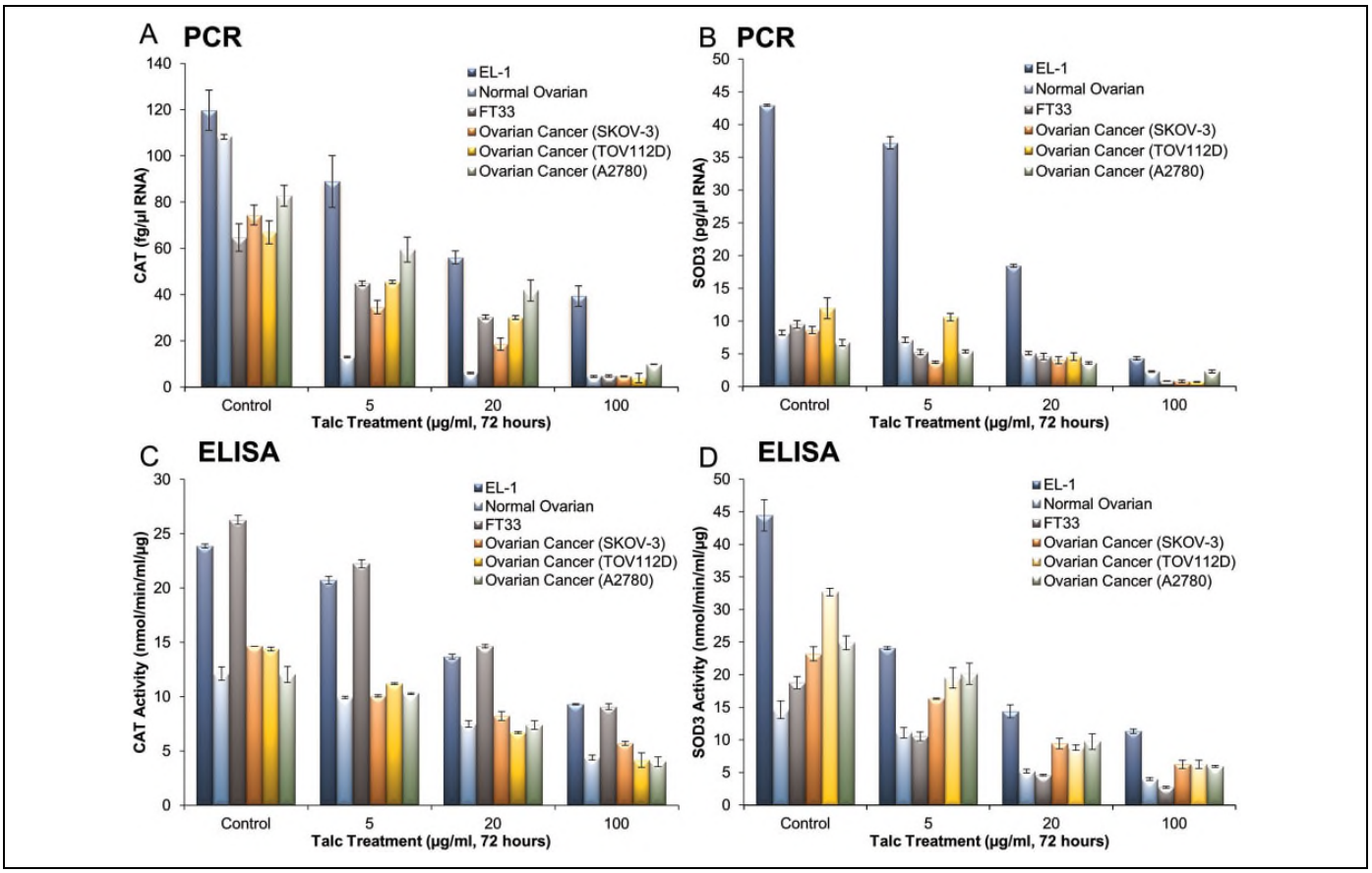

Figure 1. Decreased expression and activity of key antioxidant enzymes, CAT and SOD3. The mRNA (real-time RT-PCR) and protein/activity levels (ELISA) of CAT (A and C) and SOD3 (B and D) were determined in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls. CAT indicates catalase; SOD3, superoxide dismutase 3; mRNA, messenger RNA; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

after 72 hours of talc treatment, respectively (Figure 3). The GPX (Figure 3A and C) and GSR (Figure 3B and D) mRNA and protein levels were significantly decreased in a dose-dependent manner in talc-treated cells compared to controls ($P < .05$).

Talc Exposure Induced Known Genotype Switches in Key Oxidant and Antioxidant Enzymes

Talc treatment was associated with a genotype switch in *NOS2* from the common C/C genotype in untreated cells to T/T, the SNP genotype, in talc-treated cells, except in A2780 and TOV112D (Table 2). Additionally, the observed decrease in CAT expression and activity was associated with a genotype switch from common C/C genotype in CAT in untreated cells to C/T, the SNP genotype, in TOV112D and all normal talc-treated cells. However, there was no detectable genotype switch in CAT in A2780, SKOV3, and TOV112D (Table 2). Remarkably, there was no observed genotype switch in the selected SNP for SOD3 and GSR in all talc-treated cells. All cells, except for HOSEpiC cells, manifest the SNP genotype of *GPX1* (C/T). Intriguingly, talc treatment reversed this SNP genotype to the normal genotype (Table 2).

Talc Treatment Increased CA-125 Levels in Normal and EOC Cells

CA-125 ELISA assay was performed in protein isolated from cell media before and after talc treatment. CA-125 levels were significantly increased in a dose-dependent manner in all cells (Figure 4, $P < .05$). There was no detectable CA-125 protein in macrophages.

Talc Treatment Increased Cell Proliferation and Decreased Apoptosis

MTT cell proliferation assay was used to determine cell viability, and caspase-3 activity assay was utilized to determine apoptosis of all cell lines after 24 hours of talc treatment (Figure 5). Cell proliferation was significantly increased from the baseline in all talc-treated cells ($P < .05$), but to a greater degree in normal

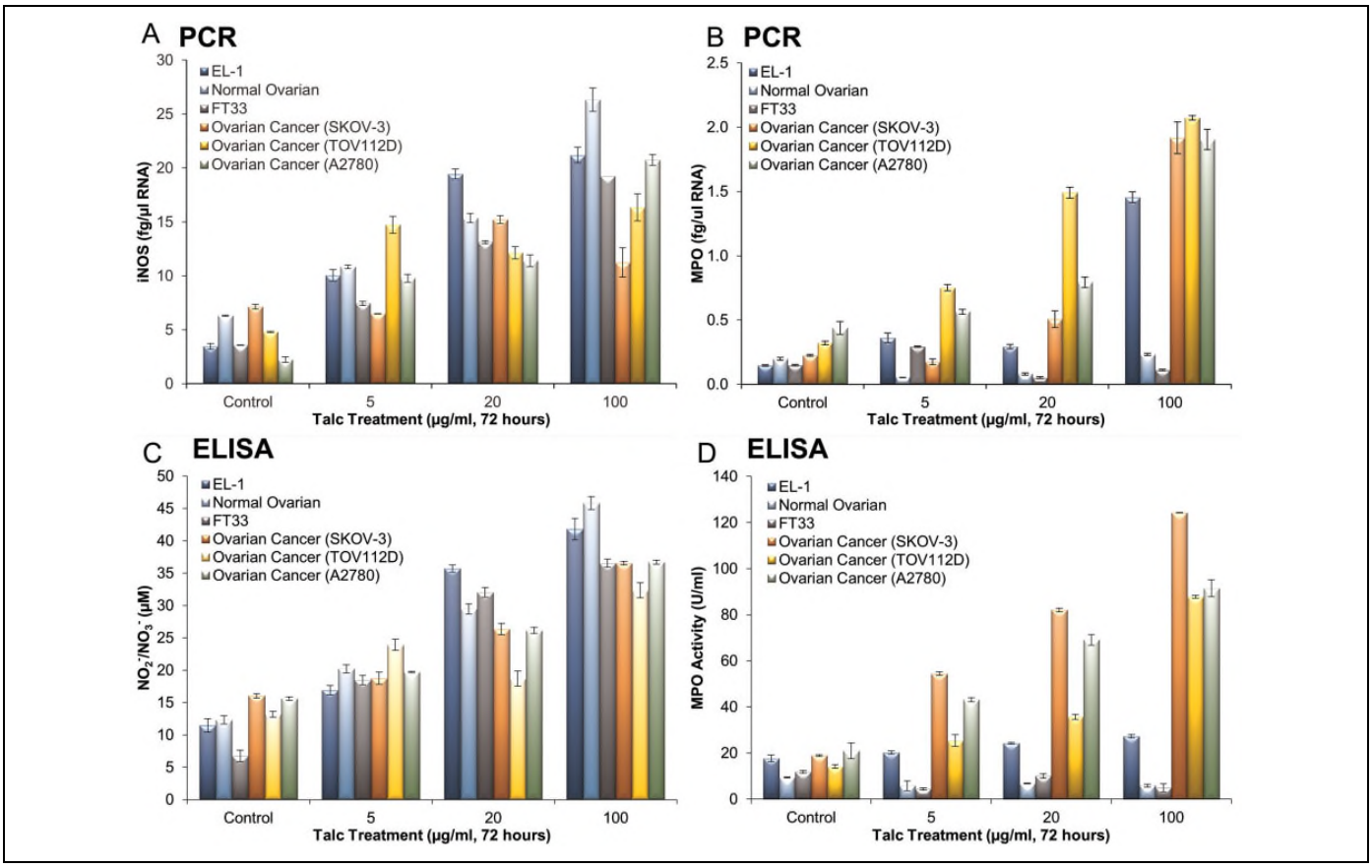

Figure 2. Increased expression and activity of key prooxidants, iNOS, NO_2^-/NO_3^-, and MPO. The mRNA (real-time RT-PCR) and protein/activity levels (ELISA) of iNOS (A and C) and MPO (B and D) were determined in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. As expected, there was no detectable MPO in normal ovarian and fallopian tube cells, and thus, talc treatment did not have any effect. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in iNOS and MPO-positive cells and in all doses as compared to controls. iNOS indicates inducible nitric oxide synthase; MPO, myeloperoxidase; mRNA, messenger RNA; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

as compared to cancer cells. As anticipated, caspase-3 was significantly reduced in cancer as compared to normal cells. Talc treatment resulted in decreased caspase-3 activity in all cells as compared to controls (Figure 6, $P < .05$), indicating a decrease in apoptosis.

Discussion

The claim that regular use of talcum powder for hygiene purpose is associated with an increased risk of ovarian cancer is based on several reports confirming the presence of talc particles in the ovaries and other parts of the female reproductive tract as well as in lymphatic vessels and tissues of the pelvis.[7-12] The ability of talc particles to migrate through the genital tract to the distal fallopian tube and ovaries is well accepted.[10] To date, the exact mechanism is not fully understood, though several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle.[8-12]

There are reports supporting the epidemiologic association of talc use and risk of ovarian cancer.[11,12] Recent studies have shown that risks for EOC from genital talc use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking. These observations suggest that estrogen and/or prolactin may play a role via macrophage activity and inflammatory response to talc. There has been debate as to the significance of the epidemiologic studies based on the fact that the reported epidemiologic risk of talc use and risk of ovarian cancer, although consistent, are relatively modest (30%-40%), and there is inconsistent increase in risk with duration of use. This observation is due, in part, to the challenges in quantifying exposure as well as the failure of epidemiological studies to obtain necessary information about the frequency and duration of usage.[11-13]

In this study, we have shown beyond doubt that talc alters key redox and inflammatory markers, enhances cell proliferation, and inhibits apoptosis, which are hallmarks of ovarian cancer. More importantly, this effect is also manifested by talc in normal cells, including surface ovarian epithelium,

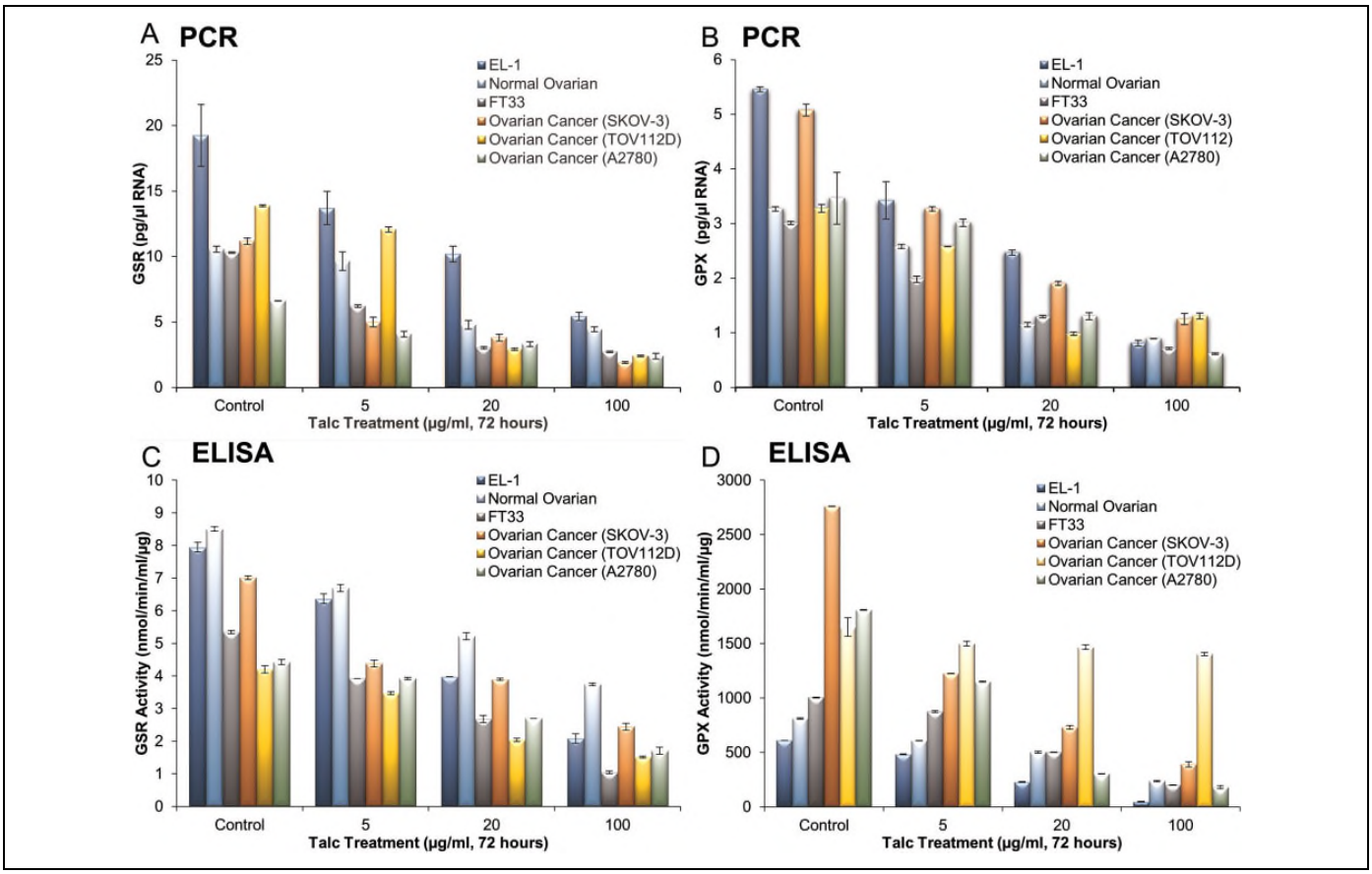

Figure 3. Decreased expression and activity of key antioxidant enzymes, GSR and GPX. The mRNA (real-time RT-PCR) and protein/activity levels (ELISA) of GSR (A and C) and GPX (B and D) were determined in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls. GSR indicates glutathione reductase; GPX, glutathione peroxidase; mRNA, messenger RNA; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

fallopian tube, and macrophages. Oxidative stress has been implicated in the pathogenesis of ovarian cancer, specifically by increased expression of several key prooxidant enzymes such as iNOS, MPO, and NAD(P)H oxidase in EOC tissues and cells as compared to normal cells indicating an enhanced redox state, as we have recently demonstrated (Figure 7).[6] This redox state is further enhanced in chemoresistant EOC cells as evident by a further increase in iNOS and NO_2^-/NO_3^- and a decrease in GSR levels, suggesting a shift toward a prooxidant state.[6] Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian.[6,14] Specifically, GPX expression is reduced in prostate, bladder, kidney, and estrogen receptor negative breast cancer cell lines, though GPX is increased in other cancerous tissues from breast.[14] Glutathione reductase levels, on the other hand, are elevated in lung cancer, although differentially expressed in breast and kidney cancer.[5,15] Similarly, CAT was decreased in breast, bladder, and lung cancer while increased in brain cancer.[16-18] Superoxide dismutase is expressed in lung, colorectal, gastric ovarian, and breast cancer, while decreased activity and expression have been reported in colorectal carcinomas and pancreatic cancer cells.[18-21] Collectively, this differential expression of antioxidants demonstrates the unique and complex redox microenvironment in cancer. Glutathione reductase is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to GSH. This enzyme is essential for the GSH redox cycle that maintains adequate levels of reduced cellular GSH. A high GSH to GSSG ratio is essential for protection against oxidative stress (Figure 5). Treatment with talc significantly reduced GSR in normal and cancer cells, altering the redox balance (Figure 3A and C). Likewise, GPX is an enzyme that detoxifies reactive electrophilic intermediates and thus plays an important role in protecting cells from cytotoxic and carcinogenic agents. Overexpression of GPX is triggered by exogenous chemical agents and reactive oxygen species and is thus thought to represent an adaptive response to stress.[15] Indeed, treatment of normal and cancer cells with talc significantly reduced GPX, which compromised the overall cell response to stress (Figure 3B and D).

Table 2. SNP Characteristics (A) and SNP Genotyping of Key Redox Enzymes in Untreated and Talc-Treated (100 μg/mL) Human Primary Ovarian Epithelial Cells (Normal Ovarian), Human Ovarian Surface Epithelial Cells (HOSEpiC), Fallopian Tube (FT33), and Ovarian Cancer (A2780, SKOV-3, TOV112D) Cell Lines (B).

	Gene (rs Number)				
	CAT (rs769217)	*NOS*$_2$ (rs2297518)	*GSR* (rs8190955)	*GPX1* (rs3448)	*SOD3* (rs2536512)
A					
MAF	0.123	0.173	0.191	0.176	0.476
SNP	C-262T	C2087T	G201T	C-1040T	A377T
Chromosome location	11p13	17q11.2	8p12	3q21.31	4p15.2
Amino acid switch	Isoleucine to Threonine	Serine to Leucine	Unknown	Unknown	Alanine to threonine
Effect on activity	Decrease	Increase	Unknown	Unknown	Decrease
B					
A2780: Control	C/C	C/C	G/G	C/T	A/A
A2780: Talc	C/C	C/C	G/G	C/C	A/A
SKOV-3: Control	C/C	C/C	G/G	C/T	A/A
SKOV-3: Talc	C/C	T/T	G/G	C/C	A/A
TOV112D: Control	C/C	C/C	G/G	C/T	A/A
TOV112D: Talc	C/T	C/C	G/G	C/C	A/A
HOSEpiC: Control	C/C	C/C	G/G	C/T	A/A
HOSEpiC: Talc	C/T	T/T	G/G	C/T	A/A
FT33: Control	C/C	C/C	G/G	C/T	A/A
FT33: Talc	C/T	T/T	G/G	C/C	A/A
Normal ovarian: Control	C/C	C/C	G/G	C/T	A/A
Normal ovarian: Talc	C/T	T/T	G/G	C/C	A/A

Abbreviation: SNP, single-nucleotide polymorphism.

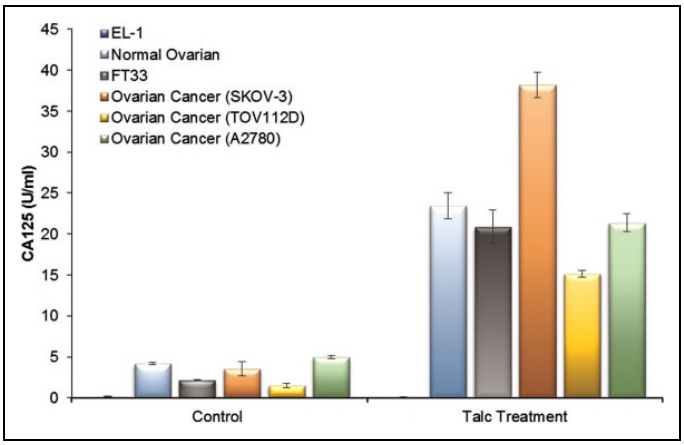

Figure 4. Increased CA-125 levels in response to talc treatment. The level of ovarian cancer biomarker CA-125 was determined by ELISA before and after 72 hours of talc treatment (100 μg/mL) in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cells. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells as compared to controls. ELISA indicates enzyme-linked immunosorbent assay.

We have previously reported that EOC cells manifest increased cell proliferations and decreased apoptosis.[6] In this study, we have shown that talc enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype. We also previously reported a cross talk between iNOS and MPO in ovarian cancer, which contributed to the lower apoptosis observed in ovarian cancer cells.[6,22] Myeloperoxidase, an abundant hemoprotein, previously known to be present solely in neutrophils and monocytes, is a key oxidant enzyme that utilizes NO produced by iNOS as a 1-electron substrate generating NO^+, a labile nitrosylating species.[6,23,24] We were the first to report that MPO was expressed by EOC cells and tissues and that silencing MPO gene expression utilizing MPO-specific siRNA induced apoptosis in EOC cells through a mechanism that involved the S-nitrosylation of caspase-3 by MPO.[22] Additionally, we have compelling evidence that MPO serves as a source of free iron under oxidative stress, where both NO^+ and superoxide are elevated.[6] Iron reacts with hydrogen peroxide (H_2O_2) and catalyzes the generation of highly reactive hydroxy radical (HO), thereby increasing oxidative stress, which in turn increases free iron concentrations by the Fenton and Haber-Weiss reaction.[6,24] We have previously highlighted the potential benefits of the combination of serum MPO and free iron as biomarkers for early detection and prognosis of ovarian cancer.[25] Collectively, we now have substantial evidence demonstrating that altered oxidative stress may play a role in maintaining the oncogenic phenotype of EOC cells. Treatment of normal or ovarian cancer cells with talc resulted in a significant increase in MPO and iNOS, supporting the role of talc in the enhancement of a prooxidant state that is a major cause in the development and maintenance of the oncogenic phenotype (Figure 2).

Furthermore, CA-125, which exists as a membrane-bound and secreted protein in EOC cells, has been established as a

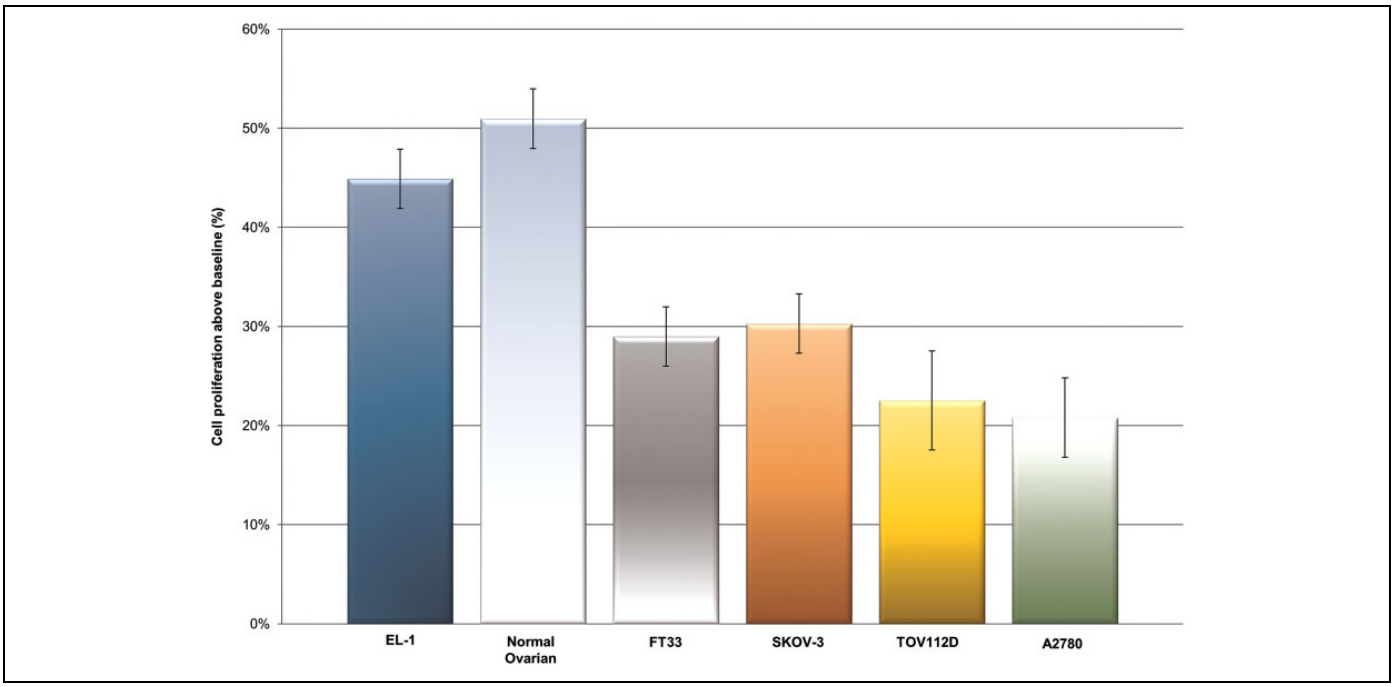

Figure 5. Increased cell proliferation in response to talc treatment. Cell proliferation was determined by MTT cell proliferation assay after 24 hours of talc treatment (100 μg/mL) in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cells. Experiments were performed in triplicate. Cell proliferation is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells as compared to controls.

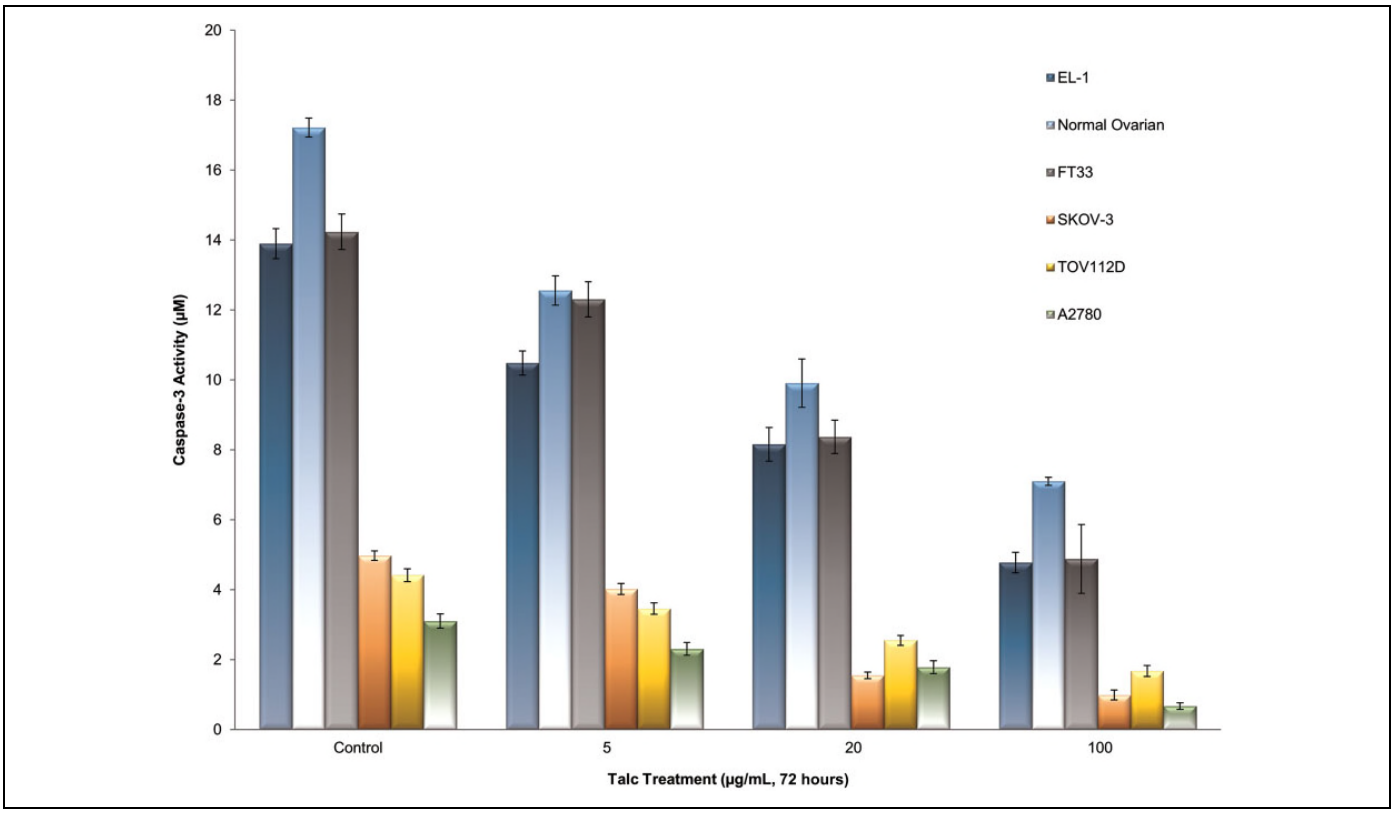

Figure 6. Decreased apoptosis in response to talc treatment. Caspase-3 activity was used to measure the degree of apoptosis in all cells. Caspase-3 activity assay was utilized to determine caspase-3 activity in macrophages (EL-1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV-3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard error. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls.

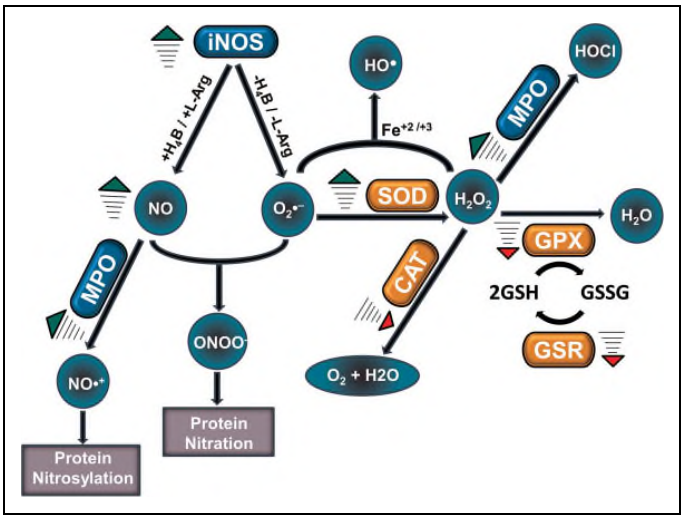

Figure 7. Epithelial ovarian cancer (EOC) cells have been reported to manifest a persistent prooxidant state as evident by the upregulation (green arrows) of key oxidants iNOS, NO, NO^+, $ONOO^-$, OH^-, O_2^+, and MPO (blue) and downregulation (red arrows) of key antioxidants SOD, CAT, GPX, and GSR (orange). This redox state was also shown to be further enhanced in chemoresistant EOC cells. In this study, talcum powder altered the redox state, as indicated by the arrows, of both normal and EOC cells to create an enhanced prooxidant state. iNOS indicates inducible nitric oxide synthase; MPO, myeloperoxidase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSR, glutathione reductase.

biomarker for disease progression and response to treatment.[2] CA-125 expression was significantly increased from nearly undetectable levels in controls to values approaching clinical significance (35 U/mL in postmenopausal women[26]) in talc-treated cells (Figure 4, $P < .05$) without the physiologic effects on the tumor microenvironment one would expect to be present in the human body, thus highlighting the implications of the prooxidant states caused by talc alone.

To elucidate the mechanism by which talc alters the redox balance to favor a prooxidant state not only in ovarian cancer cells, but more importantly in normal cells, we have examined selected known gene mutations corresponding to SNPs known to be associated with altered enzymatic activity and increased cancer risk.[6,27] Our results show that the *CAT* SNP (rs769217) resulting in decreased enzymatic activity was induced in all normal cell lines tested and in TOV112D EOC lines, but was not detected in A2780 or SKOV-3 cell lines (Table 2). Nevertheless, our results confirm a decrease in CAT expression and enzymatic activity in all talc-treated cells (Figure 1), indicating the existence of other *CAT* SNPs. The *SOD3* (rs2536512) and *GSR* (rs8190955) SNP genotypes were not detected in any cell line, yet SOD3 and GSR activity and expression were decreased in all talc-treated cells, again suggesting the presence of other SNPs. Our results have also shown that all cells, except for HOSEpiC cells, manifest the SNP genotype of *GPX1* (C/T) before talc treatment. Intriguingly, talc treatment reversed this SNP genotype to the normal genotype (Table 2). Consistent with this finding, we have previously reported that acquisition of chemoresistance by ovarian cancer cells is associated with a switch from the *GPX1* SNP genotype to the normal *GPX1* genotype.[6] It is not understood why a *GPX1* SNP genotype predominates in untreated normal and ovarian cancer cells. Our results showed that talc treatment was associated with a genotype switch from common C/C genotype in *NOS2* in untreated cells to T/T, the SNP genotype, in talc-treated cells, except in A2780 and TOV112D (Table 2). Nevertheless, our results confirm an increase in iNOS expression and enzymatic activity in all talc-treated cells (Figure 2), again suggesting the existence of other *NOS2* SNPs. Collectively, these findings support the notion that talc treatment induced gene point mutations that happen to correspond to SNPs in locations with functional effects, thus altering overall redox balance for the initiation and development of ovarian cancer. Future studies examining such SNPs are important to fully elucidate a genotype switch mechanism induced by talc exposure.

In summary, this is the first study to clearly demonstrate that talc induces inflammation and alters the redox balance favoring a prooxidant state in normal and EOC cells. We have shown a dose-dependent significant increase in key prooxidants, iNOS, NO_2^-/NO_3^-, and MPO, and a concomitant decrease in key antioxidant enzymes, CAT, SOD, GPX, and GSR, in all talc-treated cells (both normal and ovarian cancer) compared to their controls. Additionally, there was a significant increase in CA-125 levels in all the talc-treated cells compared to their controls, except in macrophages. The mechanism by which talc alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes indicate a genetic predisposition to developing ovarian cancer with genital talcum powder use.

Authors' Note

Ghassan M. Saed is also affiliated with Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI, USA.

Acknowledgment

Special thanks to Imaan Singh for her technical contributions in acquiring the data and in development of graphics.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Saed has served as a paid consultant and expert witness in the talcum powder litigation.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Exhibit J

Draft Screening Assessment

Talc
($Mg_3H_2(SiO_3)_4$)

Chemical Abstracts Service Registry Number
14807-96-6

Environment and Climate Change Canada
Health Canada

December 2018

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc. The Chemical Abstracts Service Registry Number (CAS RN[1]) for talc is 14807-96-6. This substance is among those substances identified as priorities for assessment as it met categorization criteria under subsection 73(1) of CEPA.

Talc is a naturally occurring mineral. According to information reported under section 71 of CEPA and publically available information, in 2011 talc was manufactured in Canada in quantities ranging between 50 to 75 million kg, and in 2016, approximately 100 million kg of talc was imported. In Canada talc is used in adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials; ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products, mixtures, and manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment. The major uses in Canada align with major global uses of talc. Talc is an ingredient in self-care products and is a permitted food additive. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics. High-purity talc is used in cosmetics, while lower-grade talc is used in commercial applications.

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach. The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of new PNEC values when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environment concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment. The ERC-I identified talc as having a low potential to cause ecological harm.

Considering all available lines of evidence presented in this draft screening assessment, there is a low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(*a*) or (*b*) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or

[1] The Chemical Abstracts Service Registry Number (CAS RN) is the property of the American Chemical Society, and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior written permission of the American Chemical Society.

may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Talc has been reviewed internationally by other organizations, including the International Agency for Research on Cancer (IARC) and the Danish Environmental Protection Agency. These assessments informed the human health risk assessment.

No critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and self-care products is not of concern. Inhalation exposure from industrial and commercial uses of talc was not identified to be of concern for human health given the limited number of sites producing and processing talc in Canada. Rather, the focus of the assessment is on inhalation and perineal exposure to certain self-care products containing cosmetic- or pharmaceutical-grade talc.

With respect to inhalation exposure, non-cancer lung effects were identified as a critical health effect for risk characterization on the basis of United States National Toxicology Program studies conducted with rats and mice exposed to cosmetic-grade talc. There is potential for inhalation exposure to talc powder during the use of certain self-care products (e.g., cosmetics, natural health products, non-prescription drugs formulated as loose powders). Self-care products formulated as pressed powders (e.g., face makeup) are not of concern. Margins of exposure between air concentrations following the use of dry hair shampoo and critical lung effects observed in animal studies are considered adequate to address uncertainties in the health effects and exposure databases. Margins of exposure between air concentrations following the use of loose powders (e.g., body powder, baby powder, face powder, foot powder) and critical lung effect levels observed in animal studies are considered potentially inadequate to address uncertainties in the health effects and exposure databases.

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs), a potential concern for human health has been identified.

Based on the available information, it is proposed that there is potential for harm to human health in Canada at current levels of exposure. Therefore, on the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(*c*) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

Table of Contents

List of Tables

1. Introduction

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of talc to determine whether this substance presents or may present a risk to the environment or to human health. This substance was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA (ECCC, HC [modified 2017]).

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I) approach (ECCC 2018). The ERC-I is a risk-based approach that employs multiple metrics, considering both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past predicted no-effect concentrations (PNECs) and water quality guidelines, or the derivation of a new PNEC value when required. Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model for each substance, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. Modelled and measured predicted environmental concentrations (PECs) were compared to PNECs, and multiple statistical metrics were computed and compared to decision criteria to classify the potential for causing harm to the environment.

With respect to human health, this draft screening assessment includes the consideration of information on chemical properties, environmental fate, hazards, uses, and exposures, including additional information submitted by stakeholders. Relevant data were identified up to August 2018. Empirical data from key studies, as well as results from models, were used to reach proposed conclusions. Talc has been reviewed internationally through the International Agency for Research on Cancer (IARC) Monographs Programme, United States Environmental Protection Agency (U.S. EPA), the Joint Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Danish Environmental Protection Agency (Danish EPA). Talc was also assessed by the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany and the Cosmetic Ingredient Review (CIR) Expert Panel. These evaluations and reviews were used to inform the health effects characterization in this screening assessment. This assessment focuses on health effects associated with cosmetic-grade talc and not on potential impurities, such as asbestos. Engineered nanomaterials composed of or containing talc are not explicitly considered in this assessment.

This draft screening assessment was prepared by staff in the CEPA Risk Assessment Program at Health Canada and Environment and Climate Change Canada and the Consumer Product Safety Directorate at Health Canada and incorporates input from other programs within these departments. The ecological portion of the assessment is based on the ERC-I document (published May 11, 2018), which was subject to an external peer review and a 60-day public comment period. The human health portion of

this assessment has undergone external peer review and/or consultation. Comments on the technical portions relevant to human health were received from Ms. Lopez, Ms. Super, and Ms. Jeney of Tetra Tech. Although external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

This draft screening assessment focuses on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA by examining scientific information and incorporating a weight of evidence approach and precaution.[2] This draft screening assessment presents the critical information and considerations on which the proposed conclusion is based.

2. Identity of substance

Talc (CAS RN[3] 14807-96-6) is one of the softest naturally occurring minerals, made up of magnesium, silicon, and oxygen (ChemIDplus 1993-). The term talc refers to both the pure mineral and a wide variety of soft, talc-containing rocks that are mined and used for a variety of applications (Kogel et al. 2006). Relatively pure talc ore is also referred to as steatite, and soapstone refers to impure, massive talc rock (Fiume et al. 2015).

The mineral talc is composed of triple-sheet crystalline units, consisting of two silicate sheets composed of SiO_4 tetrahedra joined by edge-link $MgO_4(OH)_2$ (Zazenski et al. 1995). These layers, held together loosely via van der Waals forces, slide over one another easily, giving talc its slippery feel and accounting for its softness (Fiume et al. 2015). The size of an individual talc platelet (i.e., a few thousand elementary sheets) can vary from approximately 1 µm to over 100 µm, depending on the conditions of formation of the deposit (Eurotalc 2017). The individual platelet size determines the lamellarity of a sample of talc. Highly lamellar talc will have large individual platelets, whereas microcrystalline talc will have small platelets. Other inorganics in place of magnesium and silicon are common in talc; for example, aluminum and iron may substitute for silicon in the tetrahedral sites, or manganese may substitute for magnesium in the octahedral positions (Zazenski et al. 1995).

[2] A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion on the basis of the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

[3] The Chemical Abstracts Service Registry Number (CAS RN) is the property of the American Chemical Society and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior written permission of the American Chemical Society.

Commercially exploited talc contains 20 to 99 % of the pure mineral (Kogel et al. 2006). Some of the most common minerals that occur with talc are carbonates (e.g., dolomite, calcite, magnesite) and chlorite (i.e., magnesium aluminum silicate) (CIR 2013). Less common minerals include quartz, mica, iron oxides, pyrite, serpentine, and amphibole. Selective mining, ore processing, and beneficiation can remove many of the impurities (Kogel et al. 2006). There is a trend towards upgrading and higher-purity talc; however, many applications require the properties of the minerals associated with talc (Kogel et al. 2006). The purity of the source talc will influence its uses.

There are different grades of talc that refer to the purity (presence of other minerals). Pharmaceutical-grade talc conforms to the United States Pharmacopeia (USP) specifications (or similar specifications); these specifications require the absence of asbestos and set limits on iron, lead, calcium, and aluminum (USP 2011). As per B.01.045 of the *Food and Drug Regulations*, when used as a food additive talc must comply with Food Chemical Codex specifications or the Combined Compendium of Food Additive Specifications, prepared by the Joint FAO/WHO Expert Committee on Food Additives, and must be free from asbestos (FAO 2006).

Cosmetic-grade talc should comply with USP standards that require a limit of 20 ppm lead and an absence of asbestos (Fiume et al. 2015). Historically, some talc source materials were contaminated with asbestos; however, in 1976 the Cosmetic Toiletry Fragrance Association (CTFA) set purity standards for cosmetic-grade talc (Fiume et al. 2015). In Canada, the *Prohibition of Asbestos and Products Containing Asbestos Regulations* to be made under CEPA 1999 will prohibit asbestos above trace levels in consumer products, including cosmetics. Health effect studies on cosmetic-grade talc cited in this assessment were considered to be free of asbestos.

Talc is milled to different particle sizes for specific commercial applications. Most talc for cosmetics and pharmaceuticals are pure 200-mesh roller-milled talc (Kogel et al. 2006). In 200-mesh talc (preferred for body powder and deodorants), the particle size distribution allows 95 to 99 % of the product to pass through a 200-mesh (74 µm) screen (Zazenski et al. 1995; Kogel et al. 2006). The finer 325-mesh talc is also used in cosmetic-, pharmaceutical-, and food-grade formulations, where 95 to 99 % of the product passes through a 325-mesh (44 µm) screen.

3. Physical and chemical properties

A summary of physical and chemical properties of talc is presented in

Table 3-1. Talc is hydrophobic and lipophilic (Kogel et al. 2006).

Table 3-1. Experimental physical and chemical property values (at standard temperature) for talc

Property	Range	Key reference
Physical state	solid, powder	HSDB 2005
Melting point (°C)	1500	Eurotalc 2017
Vapour pressure (mm Hg)	approx. 0, negligible at 20°C	OSHA 1999; NIOSH 2014
Water solubility (mg/L)	insoluble	HSDB 2005
Specific gravity (unitless)	2.58–3.83	HSDB 2005

4. Sources and Uses

Talc is a naturally occurring mineral, and there are deposits of talc in most provinces of Canada (Kogel et al. 2006). Currently, there is one producing mine (open-pit) and concentrator facility in Canada, in Penhorwood Township near Timmins, Ontario, and one micronizing facility in Timmins (Kogel et al. 2006; MAC 2016; NPRI 2018). The talc ore from the mine is approximately 45 % pure, with magnesite, magnetite, chlorite, and serpentine as the major impurities (Kogel et al. 2006). After beneficiation, this mine and micronizing facility produces talc primarily for the paper, plastics, paint, and ceramic sectors (Kogel et al. 2006). In 2017, China was the largest producer of talc, followed by India, Brazil, Mexico, and Korea (USGS 2018). The major uses of talc globally include paper, plastics, paint, ceramics, putties, and cosmetics (USGS 2000; Kogel et al. 2006; EuroTalc 2017; USGS 2018) and are aligned with Canadian uses.

On the basis of information submitted pursuant to a CEPA section 71 survey for the year 2011, talc was reported to be manufactured and imported in Canada at quantities ranging from 50 to 75 million kg (EC 2013).[4] According to the Canadian International Merchandise Trade (CIMT) database, in 2016, 99 549 000 kg of natural steatite and talc, crushed or powdered (Harmonized System, HS code 252620) and 4 656 000 kg of natural steatite and talc, not crushed, not powdered (HS code 252610) were imported into Canada (CIMT 2017).

According to information reported pursuant to a CEPA section 71 survey, results from voluntary stakeholder engagement (ECCC, HC 2017), and a search of websites from talc producers, manufactured or imported talc is used in Canada in: adhesives and sealants; automotive, aircraft, and transportation applications; building and construction materials (e.g., wood and engineered wood); ceramics; electrical and electronics; textiles; floor coverings; ink, toner, and colourants; lubricants and greases; oil and natural gas extraction applications; paints and coatings; paper and paper products,

[4] Values reflect quantities reported in response to the survey conducted under section 71 of CEPA (EC 2013). See survey for specific inclusions and exclusions (schedules 2 and 3).

mixtures, or manufactured items; plastic and rubber materials; toys, playground, and sporting equipment; and in water treatment.

Talc is a formulant in pest control products registered in Canada (Health Canada 2010, Personal communication, email from the Pest Management Regulatory Agency, Health Canada to the Risk Management Bureau, Health Canada, dated March 29, 2017; unreferenced).

Additionally, in Canada talc is on the List of Permitted Food Additives with Other Accepted Uses for limited uses in a small number of foods (Health Canada [modified 2017]). Talc can be used as a coating agent on dried legumes and rice and as a filler and dusting powder for chewing gum as per the List of Permitted Food Additives with Other Accepted Uses, incorporated by reference into its respective Marketing Authorization issued under the *Food and Drugs Act*. It may be present in food packaging materials and in incidental additives[5] used in food processing establishments (email from the Food Directorate, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada, dated March 31, 2017; unreferenced).

Talc is present in approximately 8500 self-care products.[6] Talc is marketed or approved as a non-medicinal ingredient in approximately 1600 human and veterinary drug products in Canada, including approximately 150 over-the-counter (OTC) or non-prescription products (email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017; unreferenced). Talc is listed in the Natural Health Products Ingredients Database (NHPID [modified 2018]) with a medicinal role and classified as a natural health product (NHP) substance falling under item 7 (a mineral) of Schedule 1 to the *Natural Health Products Regulations* and with a non-medicinal role (NHPID [modified 2018]). Talc is listed in the Licensed Natural Health Products Database (LNHPD) as being present as a medicinal or non-medicinal ingredient, in currently licensed natural health products in Canada (LNHPD [modified 2018]). Talc is present as a medicinal or a non-medicinal ingredient in approximately 2000 active licensed NHPs. Talc is listed as a medicinal ingredient in diaper rash products in concentrations ranging from 45 to 100 % in the Diaper Rash Monograph (Heath Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]). Talc is permitted as a medicinal ingredient in the monograph for Traditional Chinese Medicine Ingredients (Health Canada 2015).

[5] While not defined under the Food and Drugs Act (FDA), incidental additives may be regarded, for administrative purposes, as those substances that are used in food processing plants and that may potentially become adventitious residues in foods (e.g., cleaners, sanitizers).

[6] Self-care products are products available for purchase without a prescription from a doctor, and fall into one of three broad categories: cosmetics, natural health products, and non-prescription drugs.

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, talc is an ingredient in approximately 6500 cosmetic products in Canada (dated April 5, 2017, emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). Talc is considered a restricted ingredient in cosmetics.[7] The Cosmetic Ingredient Hotlist entry for cosmetics containing talc in powder form intended to be used on infants and children indicates that product labels should display text to the effect of "keep out of the reach of children" and "keep powder away from child's face to avoid inhalation that can cause breathing problems." High-purity talc (fewer impurities of other minerals) is used in cosmetics, while lower-grade talc is used in the many commercial applications mentioned above. In North America, approximately 3 to 4 % of the talc produced and sold is used in cosmetics (Kogel et al. 2006; USGS 2018).

Condoms and medical gloves are regulated as Class II medical devices in Canada under the *Medical Devices Regulations* and may be sources of exposure if talc is present as a dry lubricant. However, a 1998 study did not find talc in a small survey of condoms tested in Canada (Douglas et al. 1998). Condom standards require dry lubricants to be bioabsorbable, such as starch and calcium carbonate (WHO, UNFPA, FHI 2013). Starch is more commonly used as dry powder lubricant on condoms (Douglas et al. 1998). There was also a shift from the use of talc as a dry lubricant on medical patient examination gloves to cornstarch in the 1980s (Lundberg et al. 1997). In 2016, the U.S. Food and Drug Administration banned powdered patient examination gloves (United States 2016).

5. Potential to cause ecological harm

5.1 Characterization of ecological risk

The ecological risk of talc was characterized using the Ecological Risk Classification of Inorganic Substances (ERC-I). The ERC-I is a risk-based approach that employs multiple metrics that consider both hazard and exposure in a weight of evidence. Hazard characterization in ERC-I included a survey of past domestic and international assessment PNECs and water quality guidelines. When no suitable existing PNEC or water quality guideline was found, hazard endpoint data were collected and, dependent on data availability, either a species sensitivity distribution (SSD) or an assessment factor (AF) approach was taken to derive a new PNEC value. In the case of talc, hazard endpoint data from the Organisation for Economic Co-operation and Development

[7] Talc is described as a restricted ingredient on the List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances may contravene the general prohibition found in section 16 of the *Food and Drugs Act* (FDA), or may contravene one or more provisions of the *Cosmetic Regulations*. Section 16 of the FDA states that "no person shall sell any cosmetic that has in or on it any substance that may cause injury to the health of the user." In addition, the Hotlist includes certain substances that may make it unlikely for a product to be classified as a cosmetic under the FDA (Health Canada [modified 2018]).

Screening Information Dataset (SIDS) for synthetic amorphous silicates (OECD 2004) were identified for read across (ECCC, HC 2017) and an AF approach was used to derive a PNEC value of 40 mg/L.

Exposure profiling in ERC-I considered two approaches: predictive modelling using a generic near-field exposure model, and an analysis of measured concentrations collected by federal and provincial water quality monitoring programs. The generic near-field exposure model used input data, when available, from the National Pollutant Release Inventory (NPRI), the DSL–Inventory Update (DSL-IU), international trade data from the Canada Border Services Agency (CBSA), and third-party market research reports to generate PECs. In the case of talc, input data from the DSL-IU and CBSA were available.

Modelled PECs were compared to PNECs, and statistical metrics considering both the frequency and magnitude of exceedances were computed and compared to decision criteria to classify the potential for ecological risk as presented in ECCC (2018). The results are summarized in Table 5-1. The ERC-I identified talc as being of low ecological concern.

Table 5-1. Ecological risk classification of inorganics results for talc

Monitoring (total/extractable)	Monitoring (dissolved)	Modelling (DSL-IU)	Modelling (NPRI)	Modelling (CBSA)	Overall ERC-I score
NA	NA	Low	NA	Low	Low

Abbreviations: NA, Not Available.

6. Potential to cause harm to human health

6.1 Health effects assessment

Talc was previously reviewed internationally by the IARC, and an IARC monograph is available (IARC 2010). Additionally, talc was reviewed by the United States Environmental Protection Agency (U.S. EPA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Commission) in Germany, and the Danish Environmental Protection Agency (Danish EPA) (U.S. EPA 1992; JECFA 2006; MAK-Commission 2012; Danish EPA 2016). Talc's safety in cosmetic uses was also assessed by the CIR Expert Panel (CIR 2013; Fiume et al. 2015).

A literature search was conducted from the year prior to the most recent assessment (the 2016 Danish EPA review), i.e., from January 2015 to January 2018. No health effects studies that could impact the non-cancer risk characterization (i.e., result in different critical endpoints or lower points of departure than those stated in existing reviews and assessments) for oral, dermal, or inhalation exposures were identified. For perineal exposures, recently published literature was identified and considered in the assessment.

The health effects of talc are outlined by route of exposure in the following sections.

Toxicokinetics

Talc is poorly absorbed via the oral route of exposure. Following gavage administration of radiolabelled talc to rodents, the majority of the administered dose (AD) remained in the gastrointestinal (GI) tract and was eliminated and recovered in the faeces (≥ 95.8 % of AD) within three to four days of dosing (Wehner et al. 1977a; Phillips et al. 1978). Less than 2 % of the AD was recovered in the urine; however, this was mainly attributed to contamination from faeces during collection, with true absorption and urinary clearance expected to be even lower. At 24 hours post administration, less than 2 % of the AD remained in the carcass of hamsters; no radioactivity was detected in mouse carcasses at this time point. In rats and guinea pigs, only trace amounts of radioactivity remained in the GI tract at 10 days post administration.

As an insoluble solid, talc is not expected to be absorbed when applied to healthy and intact skin. There are no indications of dermal absorption following talc exposure (MAK-Commission 2012).

Inhalable talc particles (<10 µm) are eliminated from the respiratory tract via mucociliary clearance. In female Syrian hamsters that were administered aerosolized neutron-activated cosmetic talc at concentrations of 40 to 75 mg/m^3 (95% pure; MMAD 6.4 to 6.9 µm) over a 2-hour exposure period, 6 to 8 % of the AD was deposited into the alveoli (Wehner et al. 1977b). The biological half-life following a single exposure was estimated to be between 7 and 10 days, with complete alveolar clearance after 4 months. There was no translocation of talc from the respiratory tract to the liver, kidneys, ovaries, or other parts of the body. Lung clearance was noted to be longer in other species. The Danish EPA (2016) noted that talc, including the respirable fraction (< 4 µm), is not absorbed following inhalation, but is retained in the lung tissue. They further stated that lung burdens were proportional to respired concentrations, and clearance became impaired with increasing exposures. Pulmonary retention half-lives for talc particles in the lungs of rats from a chronic inhalation study were estimated to be as long as 300 days (Oberdorster 1995). Other authors (Pickrell 1989; MAK-Commission 2012) noted similar findings indicating that with repeat exposures, alveolar clearance in rats may be impaired at concentrations of only 2 mg talc/m^3 air.

Talc particles have been observed and detected in the ovaries of humans (Heller et al. 1996a, 1996b), and perineal exposure to talc has also been associated with a presence of talc in lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996b; Cramer et al. 2007). Migration of talc particles from the vagina to the ovaries has been identified as a plausible explanation of these findings (Henderson et al., 1986), and retrograde movement of talc particles in humans through the reproductive tract to the ovaries has been suggested (Heller et al. 1996b; Cramer et al. 2007). Inert particles with the same size as talc (5 to 40 µm in diameter) and placed in the vagina can be transported to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979).

According to a review by the MAK-Commission (2012), there are no indications of metabolism via typical degradation pathways from which toxicologically relevant degradation products may develop.

Health Effects

Oral route of exposure

Talc was considered be of low concern with respect to human health via oral exposure. Repeated-dose testing with talc in animals did not produce any adverse effects via oral exposure with respect to repeated-dose toxicity, carcinogenicity, reproductive/developmental toxicity, or mutagenicity (Gibel et al. 1976; Wagner et al. 1977; NTP 1993; IARC 2010; Danish EPA 2016).

Talc has not been shown to produce adverse effects when ingested orally; as a result, the use of talc in various tablet formulations was not considered hazardous via the ingestion route (Hollinger 1990; U.S. EPA 1992).

In addition, the Commission of the European Communities' report on Dietary Food Additive Intake in the European Union identified talc as having an Acceptable Daily Intake (ADI) of "not-specified." The JECFA has also assessed talc and assigned an ADI as "not specified" due to the lack of toxicity from oral exposure. The substance was considered not to be a hazard to human health at oral intake levels noted in total diet surveys, which represent the majority of the sources of oral exposure for this substance (IARC 1987; EU [modified 2001]). Furthermore, talc is considered as "generally recognized as safe" when used as a food additive in the United States (U.S. FDA GRAS list) without being subject to pre-market approval requirements (U.S. FDA 2015; 2016).

Dermal route of exposure

There are limited data available on repeated-dose studies via dermal exposure to talc (Danish EPA 2016). In the available literature, only one repeated-dose dermal toxicity study was identified (Wadaan 2009). Severe limitations were noted for this study, including a lack of information on the test substance and the dose applied, as well as a lack of detail regarding the test animals. Skin dryness and erosion were noted; however, application sites were shaved, indicating that talc may have been applied to broken skin. As such, the results of this study were not considered appropriate to inform the characterization of health effects via dermal exposure. Additionally, there were no indications of irritation, sensitization, or dermal absorption following exposure to unabraded and/or non-diseased skin (MAK-Commission 2012). A three-day occlusive application of pharmaceutical-grade talc did not show any signs of irritation in 5 human volunteers (Frosch and Kligman 1976, as reported in MAK-Commission 2012).

Case reports, however, do indicate that the application of talc to diseased or broken skin can cause the formation of granulomas, particularly if the talc particles have a large diameter (MAK-Commission 2012; CIR 2013; Fiume et al. 2015). Granulomas have

been observed in the umbilical regions of infants, in the testes, on the vocal cords, in the urinary tract, and during phlebectomies following contact with talc-powdered surgical gloves (Ramlet 1991, Simsek et al. 1992, as reported in MAK-Commission 2012). As a result, the CIR concluded that "talc should not be used on skin where the epidermal barrier is removed or on skin that has greater than first degree burns."

Although dermal contact with talc is expected from the use of various products available to consumers, talc is a solid powder that is insoluble in water (Table 3-1). As a result, it cannot readily penetrate intact skin, and therefore systemic absorption through the skin is not expected. Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), a dermal health effects endpoint has not been identified for talc.

Inhalation route of exposure

Human studies

The Danish EPA (2016) noted that talc is not absorbed via inhalation. Rather, particles are retained in the lung, and lung burdens increase proportionally with exposure concentrations or frequency. The report detailed epidemiological data that noted mortalities in workers due to lung diseases, following exposures to talc. However, it was stated that there was no increase in the lung cancer rate in talc millers in the absence of exposure to carcinogens. A recent meta-analysis by Chang and colleagues (2017) reported a positive association with lung cancer in workers exposed to talc; however, co-exposure to other hazardous materials in the workplace and smoking were not adequately accounted for.

The chronic inhalation of talc leads to lung function disorders and fibrotic changes in humans. Since talc particles are persistent, particles accumulate in human lung tissue. This accumulation may lead to both an impairment of the self-purification function (reduced ability to fight infections) and inflammatory changes and fibrosis. Talc particles may be enclosed in a foreign-body granuloma as the result of an inflammatory reaction. The immobility of the macrophages, which is restricted by the phagocytized talc particles, leads to changes in the function of these cells and subsequently to chronic inflammatory reactions (Gibbs et al. 1992).

In humans, there are reports of pure talc-induced pneumoconiosis or talcosis following inhalation exposure to talc. Talcosis has been reported to occur in miners, millers, rubber workers, and other occupational groups exposed to talc without asbestos or silica (Vallyathan and Craighead 1981; Feigin 1986; Gibbs et al. 1992; Akira et al. 2007). Specifically, a recent longitudinal survey of French and Austrian talc workers found that the prevalence of small radiological opacities and decreases in lung function parameters were related to cumulative exposure. The mean estimated talc dust concentration during the mean duration of follow-up (14.5 years) was 1.46 mg/m^3 (Wild et al. 2008). Case reports indicate that patients present with non-specific complaints, including progressive exertional dyspnea, dry or productive cough, with indications of

lung lesions (Marchiori et al. 2010; Frank and Jorge 2011). Talcosis has been shown to occur in children and adults, with symptoms that developed shortly after acute to short-term exposure or up to 10 years later (Patarino et al. 2010; Shakoor et al. 2011). Inhalation of talc has been known to cause pulmonary effects, even following single acute exposures, as reported in a 10-year-old child who had a history of a single exposure to talc at two years of age (Cruthirds et al. 1977). Another case report detailed a seven-year-old child who developed asthma and reduced lung function after a single exposure event (Gould and Barnardo, 1972). Additionally, a 52-year-old woman who used baby talcum powder regularly at least twice a day (usually after bathing for personal hygiene and habitually applying it to her bed sheets nightly) for 20 years was reported to have dyspnea, along with a persistent dry cough and unintentional rapid weight loss. A radiographic exam noted evidence of interstitial lung disease with fibrosis (Frank and Jorge 2011).

Other relevant case reports include the case of a 55-year-old woman, occupationally exposed to talc as a dusting agent on packed rubber balls from 1958 to 1968, who was reported to develop dyspnea during the first five years after exposure (Tukiainen et al. 1984); and a 62-year-old woman occupationally exposed to talc for five years who was reported to have progressive lung fibrosis for more than 40 years (Gysbrechts et al. 1998).

Animal studies

In a repeated-exposure study conducted by the U.S. National Toxicology Program (NTP), groups of F334/N rats were exposed to aerosolized talc via the inhalation route of exposure. Test animals were exposed for 6 hours per day, 5 days per week, for up to 113 weeks (males) or up to 122 weeks (females) to aerosols of 0, 6, or 18 mg/m^3 talc (49 or 50 males per group, 50 females per group) (NTP 1993). Mean body weights of rats exposed to 18 mg/m^3 talc were slightly lower than those of controls after week 65. No clinical observations were attributed to talc exposure. Absolute and relative lung weights of male and female rats exposed to 18 mg/m^3 talc were significantly greater than those of controls. Inhalation exposure produced a spectrum of inflammatory, reparative, and proliferative processes in the lungs. Granulatomous inflammation, which was evident as early as 6 months (first histopathological examination), occurred in nearly all exposed rats, and the severity increased with exposure duration and concentration. Hyperplasia of the alveolar epithelium and interstitial fibrosis occurred in or near the foci of inflammation in many exposed rats, while squamous metaplasia of the alveolar epithelium and squamous cysts were also occasionally seen. Accumulations of macrophages (histiocytes), most containing talc particles, were found in the peribronchial lymphoid tissue of the lung and in the bronchial and mediastinal lymph nodes. In exposed male and female rats, there was a concentration-related impairment of respiratory function, beginning at 11 months, which increased in severity with increasing exposure duration. The impairment was characterized by reductions in lung volume (total lung capacity, vital capacity, and forced vital capacity), lung compliance, gas exchange efficiency (carbon monoxide diffusing capacity), and non-uniform intrapulmonary gas distribution (NTP 1993).

In female rats at 18 mg/m^3 talc, the incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) were significantly greater than those of controls (NTP 1993). The incidences of lung neoplasms in exposed male rats were similar to those in controls. Adrenal medulla pheochromocytomas (benign, malignant, or complex [combined]) occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m^3 talc groups were significantly greater than those of controls (NTP 1993).

The NTP (1993) concluded that there was some evidence of carcinogenic activity of talc in male rats on the basis of an increased incidence of benign or malignant pheochromocytomas of the adrenal gland. The NTP also concluded that there was clear evidence of carcinogenic activity of talc in female rats on the basis of increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland.

In a subsequent symposium, experts from the NTP, along with academic, industry, and government experts re-examined the results of the chronic inhalation studies. The general consensus from the expert panel was that the highest dose tested (18 mg/m^3) exceeded the Maximum Tolerated Dose (MTD) and as such, the neoplasms noted were not relevant to human health risk assessment (Carr 1995). A similar conclusion was rendered by Warheit et al. (2016). In addition, the Danish EPA (2016) and the MAK-Commission attributed lung tumours in female rats to the general particle effect of granular biopersistent dusts, which manifests as tumours in rodents only, and not the specific effect of the talc particles. They also attributed the pheochromocytomas to an increase in cell proliferation due to hypoxia, which was considered to be a high-dose effect (MAK-Commission, 2012).

A chronic, repeated-exposure study was conducted in B6C3F1 mice via the inhalation route of exposure (NTP 1993). Test animals were exposed for 6 hours per day, 5 days per week, for up to 104 weeks to aerosols of 0, 6, or 18 mg/m^3 talc (47 to 49 males per group, 48 to 50 females per group). Survival and final mean body weights of male and female mice exposed to talc were similar to those of the controls. There were no clinical findings attributed to talc exposure. Inhalation exposure of mice to talc at both concentrations was associated with chronic active inflammation and the accumulation of macrophages, which contained talc, in the lung. In contrast to rats, hyperplasia of the alveolar epithelium, squamous metaplasia, or interstitial fibrosis were not associated with the inflammatory response in mice, and the incidences of lung neoplasms in exposed and control groups of mice were similar. Accumulations of macrophages (histiocytes) containing talc particles were also present in the bronchial lymph node. The critical-effect level and corresponding health effects endpoint was a lowest observed adverse effect concentration (LOAEC) of 6 mg/m^3 for non-cancer lung effects (NTP 1993).

Doses used in the NTP chronic studies were selected on the basis of the results of a 4-week inhalation study (1993) in which rats and mice were exposed to talc at 0, 2, 6, or 18 mg/m^3, 6 hours a day, 5 days a week. Lung burdens were noted to be increased in a

dose-dependent manner, with overload noted by the study authors at 6 and 18 mg/m^3 in rats but not at any dose in mice. In both species (mice and rats), a minor macrophage infiltration of lung tissue was the only health effect noted in the high-dose animals, while animals in the mid- and low-dose groups were without treatment-related effects.

In a review of the NTP studies, Oberdorster (1995) revisited the lung deposition data and particle accumulation kinetics in the lungs of rats and mice in those studies, demonstrating that impaired clearance and lung overload was reached at 6 mg/m^3 and above, for both sexes, in rats and mice.

A no-observed adverse effect concentration (NOAEC) of 2 mg/m^3 was derived from the 4-week study, on the basis of increased lung burden and impaired clearance at a LOAEC of 6 mg/m^3 following 4-weeks of dosing, which led to non-cancer lung lesions at this concentration when the duration of dosing was extended. Granulatomous inflammation and alveolar epithelial hyperplasia were noted at a 6 month interim sacrifice in the chronic rat inhalation study, with interstitial fibrosis and impaired lung function noted in some animals at 11 months. As noted previously, following a single exposure in rats, the biological half-life for ciliary clearance was between 7 and 10 days, indicating that previous exposure would not have cleared prior to subsequent exposures, leading to a build-up in lung tissue. A re-examination of the NTP lung burden data by Oberdorster (1995) estimated that lung retention half-lives of talc particles were between 250 and 300 days in the rat chronic study. On the basis of this information, it was considered relevant to combine the NTP studies for the derivation of an appropriate point of departure for lung effects associated with repeated inhalation exposures.

The Danish EPA (2016) used the LOAEC of 6 mg/m^3 from the chronic NTP studies (mice and rats) and a NOAEC of 1.5 mg/m^3 for talc-induced non-cancer lung effects in the longitudinal survey of French and Austrian talc workers (Wild et al. 2008) to establish a health-based quality criterion for ambient air (QC_{air}) of 0.004 mg/m^3.[8]

While human occupational studies and case studies are available, these studies do not provide accurate measures of exposure for use in risk characterization. However, human studies do note a similar range of lung effects and disease as animal models. As such, results from the animal studies noted above were selected for the non-cancer risk characterization. On the basis of the NTP studies with rats and mice exposed to cosmetic-grade talc, a NOAEC of 2 mg/m^3 for non-cancer lung effects is considered to be appropriate for the inhalation route of exposure for short- or long-term use (given the long half-life and slow lung clearance of talc from the lungs, even episodic exposures would be expected to increase lung load). The NOAEC of 2 mg/m^3 was adjusted according to U.S. EPA guidance on inhalation risk assessment for a comparison with

[8] The health-based quality criterion in ambient air (QC_{air}) is a reference concentration that refers to the maximum permissible contribution to air from industrial sources.

exposure estimates (U.S. EPA 1994, 2009).[9] The adjusted NOAEC for non-cancer effects is 0.36 mg/m^3.

Perineal exposure to talc

The IARC has classified perineal use of talc-based body powder as "possibly carcinogenic to humans" (Group 2B) on the basis of limited evidence in humans. The analyzed case-control studies found a modest but consistent increase in risk, although bias and confounders could not be ruled out. The IARC Working Group concluded that, taken together, the epidemiological studies provide limited evidence in humans of an association between perineal use of talc-based body powder and an increased risk of ovarian cancer, although a minority of the Working Group considered the evidence inadequate because the exposure-response was inconsistent and the cohort analyzed did not support an association (IARC 2010).

The CIR Expert Panel (2013) determined that there is no causative relationship between cosmetic use of talc in the perineal area and ovarian cancer, and further concluded that talc is safe in the practices of use and concentration described in the CIR safety assessment. Issues noted by the CIR included a lack of consistent statistically significant positive associations across all studies; small risk ratio estimates; a failure to rule out other plausible explanations such as bias, confounders, and exposure misclassifications; and a lack of evidence from studies of occupational exposures and animal bioassays (CIR 2013; Fiume et al. 2015).

Animal studies

Rodents are poor experimental models for perineal studies for a number of reasons. Ovulation in rodents occurs only or mainly during the breeding season, and rodent ovaries are variously enclosed in an ovarian bursa in comparison to human ovaries. Ovarian epithelial tumours are also rare in these animals (Taher et al. 2018). Ovarian tumours do occur in some strains of mice and rats; however, the low incidence and/or the length of time required for the appearance of tumours renders them poorly feasible for experimental studies of ovarian carcinogenesis (Vanderhyden et al. 2003). On account of the limitations detailed above, in addition to the challenges posed by exposing animals via the perineal route, animal data are very limited; one single-dose study and one short-term repeated-dose study were available (Hamilton et al. 1984;

[9] This adjustment was made according to guidance and equations outlined in the U.S. EPA Supplemental Guidance for Inhalation Risk Assessment (US EPA 2009) and the U.S. EPA Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA 1994). Adjustment of duration to a continuous exposure scenario is done through the use of Equation 1 from U.S. EPA 2009 where the NOAEL[ADJ] = E × D × W, whereby the NOAEL[ADJ] (mg/m^3) = the no-observed adverse effect level (NOAEL) adjusted for the duration of the experimental regimen; E (mg/m^3) = the NOAEL or analogous exposure level observed in the experimental study; D (h/h) = the number of hours exposed/24 hours; and W (days/days) = the number of days of exposure/7 days. The NOAEC[ADJ] = 2 mg/m^3 × 6h/24h × 5d/7d = 0.36 mg/m^3

Keskin et al. 2009). No chronic or carcinogenicity animal studies on perineal exposure of talc were located in the literature.

A single injection of talc (in saline) into the bursa around the ovaries of rats showed foreign-body granulomas with confirmation of the presence of talc (Hamilton et al. 1984). Daily perineal or intravaginal application of talc (in saline) to rats for 3 months produced evidence of foreign-body reaction and infections; in addition, an increase in the number of inflammatory cells were found in all genital tissues. While no cancer or pre-cancer effects were observed, Keskin and colleagues (2009) noted that the study duration may have been too short to note these types of effects.

Human studies

Several meta-analyses of available epidemiological data have been published; some very recently (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). These studies have consistently reported a positive association with ovarian cancer and perineal talc exposure. Taher and colleagues (2018) identified 27 studies (24 case-control and 3 cohort) for a meta-analysis; ever versus never perineal use of talc and the risk of ovarian cancer resulted in a statistically significant pooled odds ratio (OR) of 1.28 (see Table 6-1). Other published meta-analyses have demonstrated similar results, with ORs ranging from 1.22 to 1.35 (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018).

Table 6-1. Available human epidemiological studies investigating the association of perineal use of talc and ovarian cancer (Taher et al. 2018, in preparation)

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
Case-control	686 (235)	Possible association in subgroup	Not included	Booth et al. 1989
Case-control	1014 (450)	Positive association	1.42 [1.08, 1.87]	Chang and Risch 1997
Case-control	336 (112)	Positive association in subgroup	Not included	Chen et al. 1992
Case-control	735 (313)	Positive association	1.60 [1.10, 2.33]	Cook et al. 1997
Case-control	430 (215)	Positive association	1.92 [1.27, 2.90]	Cramer et al. 1982
Case-control	4141 (2041)	Positive association	1.32 [1.15, 1.51]	Cramer et al. 2016
Case-control	3187 (1385)	Positive association	1.36 [1.14, 1.62]	Gates et al. 2008

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
Case-control	305 (153)	No association	2.49 [0.94, 6.60]	Godard et al. 1998
Case-control	1684 (824)	Positive association	1.30 [1.10, 1.54]	Green et al. 1997
Case-control	274 (116)	No association	1.10 [0.70, 1.73]	Harlow and Weiss 1989
Case-control	474 (235)	Positive association in subgroup	1.50 [1.00, 2.25]	Harlow et al. 1992
Case-control	306 (135)	No association	0.70 [0.40, 1.22]	Hartge et al. 1983
Case-control	2704 (902)	Positive association	1.40 [1.16, 1.69]	Kurta et al. 2012
Case-control	225 (46)	No association	1.15 [0.41, 3.23]	Langseth and Kjaerheim 2004
Case-control	3085 (1576)	Positive association in subgroup	1.17 [1.01, 1.36]	Merritt et al. 2008
Case-control	1354 (249)	Positive association in subgroup	1.37 [1.02, 1.84]	Mills et al. 2004
Case-control	2143 (1086)	No association	1.06 [0.85, 1.32]	Moorman et al. 2009
Case-control	2134 (767)	Positive association in subgroup	1.50 [1.10, 2.05]	Ness et al. 2000
Case-control	123 (77)	Possible association	1.00 [0.20, 5.00]	Rosenblatt et al. 1992
Case-control	2125 (812)	Possible association	1.27 [0.97, 1.66]	Rosenblatt et al. 2011
Case-control	1329 (584)	Positive association	1.44 [1.11, 1.87]	Schildkraut et al. 2016
Case-control	389 (189)	No association	1.05 [0.28, 3.94]	Tzonou et al. 1993
Case-control	727 (188)	Possible association	1.45 [0.81, 2.60]	Whittemore et al. 1988
Case-control	1155 (462)	No association	1.00 [0.80, 1.25]	Wong et al. 1999
Case-control	1297 (609)	Positive association	1.53 [1.13, 2.07]	Wu et al. 2009
Case-control	4092 (1701)	Positive association in	1.46 [1.27, 1.68]	Wu et al. 2015

Study type	Total sample size (no. of cases)	Study conclusion	OR [95% CI]	Reference
		subgroup		
Cohort	108870 (797)	Possible association in subgroup	Not included	Gates et al. 2010
Cohort	78630 (307)	Possible association in subgroup	1.09 [0.86, 1.38]	Gertig et al. 2000
Cohort	41654 (154)	No association	0.73 [0.44, 1.21]	Gonzalez et al. 2016
Cohort	61285 (429)	No association	1.12 [0.92, 1.36]	Houghton et al. 2014

Abbreviation: CI, confidence interval.

Mode of action

The etiology of most ovarian tumours, in general, has not been well established. There are a number of different tumour types with characteristic histologic features, distinctive molecular signatures, and disease trajectories. Moreover, these tumours are heterogeneous, and they can arise from different tissues of the female reproductive tract, including the fallopian tube epithelium (National Academy of Sciences, Engineering, and Medicine 2016).

With respect to talc specifically, local chronic irritation leading to an inflammatory response is one possible mechanism of tumour progression that is frequently hypothesized (Muscat and Huncharek 2008; Penninkilampi and Eslick 2018; Taher et al. 2018). It is known that persistent indications of inflammation (including C-reactive protein, tumour necrosis factor, and other inflammatory markers) are detected in the blood of women prior to a diagnosis of ovarian tumours (Trabert et al. 2014). Increases in the number of inflammatory cells were found in all genital tissues of rats intravaginally exposed to talc for 3 months (Keskin et al. 2009). There is support for an association of inflammation and increased risk of ovarian cancer (National Academy of Sciences, Engineering and Medicine 2016; Rasmussen et al. 2017).

Talc particles were detected in the ovaries of rats that received intrauterine instillations of talc, and to a lesser extent in those that were dosed intravaginally with talc (Henderson et al. 1986). No translocation of talc into the ovaries was detected after single or multiple intravaginal applications of talc to rabbits (Phillips et al. 1978) or to monkeys (Wehner et al. 1986).

Talc particles were identified in 10 of 13 human ovarian tumours but were also found in 5 of 12 “normal” ovarian tissues removed from patients with breast cancer (Henderson et al. 1971). Ovaries from 24 patients undergoing incidental oophorectomy were examined; 12 women reported frequent perineal talc use, and the other 12 women were

non-users. Talc particles were detected in all 24 cases (both ever- and non-users) (Heller et al. 1996b). Wehner (2002) attributed the talc in the never users to (a) possible sample contamination, because some studies using negative controls resulted in particle counts similar to the test sample; and/or (b) possible false positives due to the use of a single radioactive tracer. To explain why talc is present in the never users, Heller and colleagues (1996b) hypothesized that talc use during diapering could contribute to the ovarian particle burden.

Translocation of other inert particles, similar in size to talc, has also been studied. A study in monkeys did not show any translocation of carbon black particles when a suspension was placed in the vaginal posterior fornix (Wehner et al. 1985). However, retrograde migration was detected when rabbits were administered a lubricant powder intravaginally (Edelstam et al. 1997). Other authors have noted similar transportation of particles to the upper genital tract (Egli and Newton 1961; De Boer 1972; Venter and Iturralde 1979). There are also some indications that particles can migrate from the vagina to the upper reproductive tract in humans (Egli and Newton 1961; Venter and Iturralde 1979; Heller et al. 1996a,b), and perineal exposure to talc has also been associated with a presence of talc in the lymph nodes and ovaries of women diagnosed with ovarian cancer (Heller et al. 1996a,b; Cramer et al. 2007).

Another possible mode of action that is hypothesized in the scientific literature is immune-mediated. It has been suggested that talc particles need not reach the ovaries but only need to reach the lower genital tract where talc could trigger changes (such as the production of heat shock proteins and/or decreased levels of antibodies) that could contribute to ovarian cancer (Cramer et al. 2005; Muscat et al. 2005). Human mucin 1 (MUC1) is expressed in high levels by ovarian cancer. Mucins are proteins involved in the formation of mucous barriers on epithelial surfaces (Gendler and Spicer 1995). Anti-MUC1 antibodies may have a protective effect; patients generate immunity against MUC1 produced by their tumours (Cramer et al. 2005). The Cramer et al. (2005) study used an enzyme-linked immunosorbent assay to measure anti-MUC1 antibody in women (controls; n = 721) to determine the factors that predict the presence of antibodies. It was found that the use of talc in the perineal area was associated with significantly decreased levels of antibodies to MUC1 (Cramer et al. 2005).

The most recent meta-analysis (Taher et al. 2018) employed the Hill criteria (Hill 1965) to assess the epidemiological evidence of a causal relationship. The Hill considerations are a set of factors (i.e., strength, consistency, specificity, temporality, biological gradient, biological plausibility, and coherence). These considerations form a framework for evaluating evidence in humans to help determine whether observed associations are causal (Hill 1965; Cogliano et al. 2004; US EPA 2005; Health Canada 2011; Fedak et al. 2015). Each factor, as reported in Taher et al. (2018), is elaborated upon below.

Strength: Of the 30 epidemiological studies examined by Taher et al. (2018), 15 case-control studies reported a positive association with statistical significance; 6 of these 15 had an OR of 1.5 or greater. Similarly, Penninkilampi and Eslick (2018) and Berge and colleagues (2018) each assessed 27 epidemiological studies and respectively

determined 14 and 13 case-control studies as reporting a positive association with statistical significance. In both cases, 5 of these studies had an OR of 1.5 or greater. Terry and colleagues (2013) only pooled 8 case-control studies; 5 of the 8 (63%) had a statistically significant positive association.

The individual cohort studies did not show a statistically significant association between perineal talc use and ovarian cancer (Berge et al 2018; Penninkilampi and Eslick 2018; Taher et al 2018). However, there was a positive association, with statistical significance, specific to invasive serous-type ovarian cancer in the cohort studies (OR = 1.25) (Penninkilampi and Eslick 2018). Given the long latency for ovarian cancer, the follow-up periods may not have been sufficient to capture all the cases for the individual cohort studies. Also, given the rarity of ovarian cancer, many of the available human studies may not be sufficiently powered to detect a low OR. Sample sizes were not large enough to detect a 20 to 30 % increase in risk; a group of over 200 000 women would need to be followed for over 10 years in order to detect a 20% (above background) increased risk with statistical significance (Narod 2016). With larger sample sizes, more individual studies may have demonstrated stronger associations.

Consistency: Several meta-analyses conducted over the past 15 years calculated similar ORs and resulted in similar conclusions; that there is a small yet consistent and statistically significant increased risk for ovarian cancer with perineal talc use (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al 2018). The epidemiological studies examined in these meta-analyses were conducted over different periods in time (across more than four decades), among different ethnicities, and spanned many geographical areas worldwide (Taher et al. 2018).

Specificity: Although there are many other risk factors for ovarian cancer (e.g., increased age, family history of cancer, obesity, nulliparity) (National Academy of Sciences, Engineering, and Medicine 2016), perineal talc exposure is specifically associated with cancer of the ovary and not other organs (Taher et al. 2018).

Temporality: In all case-control studies reporting positive outcomes, the participants recalled that exposure to talc preceded the reported outcome. However, in the cohort studies (reporting a lack of positive association), it is not known whether the follow-up period was adequate to detect a potential association between perineal talc exposure and ovarian cancer (Taher et al. 2018).

Biological gradient: There is a lack of an available exposure-effect relationship in the human epidemiological data. Many of the studies only assessed a single-dose level (ever versus never users). Furthermore, data with respect to the types of powder used by subjects or the amounts applied were not presented, and therefore a relationship between the concentration/dose of talc in the powder and the incidence of ovarian cancer could not be investigated. Taher and colleagues (2018) isolated seven studies that provided some evidence of increased risk of ovarian cancer with increasing perineal applications of talc; however, none demonstrated both a clear dose-response

trend and statistical significance (Whittemore et al. 1988; Harlow et al. 1992; Mills et al. 2004; Wu et al. 2009; Rosenblatt et al. 2011; Cramer et al. 2016; Schildkraut et al. 2016).

Biological plausibility: Particles of talc are hypothesized to migrate into the pelvis and ovarian tissue, causing irritation and inflammation. The presence of talc in the ovaries has been documented (Heller et al. 1996b). This evidence of retrograde transport supports the biologic plausibility of the association between perineal talc application and ovarian exposure; however, the specific mechanism(s) and cascade of molecular events by which talc might cause ovarian cancer have not been identified (Taher et al. 2018).

Coherence: Multiple case-control studies reported a lower risk of ovarian cancer in women who underwent pelvic surgery or tubal ligation (which disrupts the pathway and movement of talc from the lower to the upper genital tract) and suppressed ovulation (as cited by Taher et al. 2018: Cramer et al. 1982, 2016; Whittemore et al. 1988; Rosenblatt et al. 1992; Green et al. 1997; Wong et al. 1999; Mills et al. 2004). As noted in Penninkilampi and Eslick (2018), the main reductions in cancer incidence with tubal ligation were for serous and endometrial tumour types but not for mucinous or clear-cell tumours. Thus, tubal ligation is only effective in reducing the incidence of the same tumour types noted to be associated with perineal talc use.

The most recent meta-analysis detailed above (Taher et al. 2018), and consistent with the Hill criteria, suggests a small but consistent statistically significant positive association between ovarian cancer and perineal exposure to talc. Further, available data are indicative of a causal effect. A clear point of departure could not be derived from the available literature; consequently, hazard characterization is qualitative in nature.

6.2 Exposure assessment

This exposure assessment focuses on routes of exposure where critical effects have been identified; namely, non-cancer lung effects following inhalation of insoluble respirable particles of talc, and an association with ovarian cancer following perineal exposure to talc.

6.2.1 Environmental media, food and drinking water

Talc is a naturally occurring mineral, and there are several deposits in Canada (Kogel et al. 2006). Currently, there is one operating open-pit mine and concentrator along with an operating mill (MAC 2016); however, no talc concentration data in ambient air or around open-pit talc mines and processing facilities have been reported. Although particulate matter (PM) information for inhalable and respirable particles is available in the vicinity of these facilities (NPRI 2018), these data were not used in the exposure assessment as PM released from facilities is expected to contain a mixture of substances, hence the concentration would not reflect talc exposure from this source. However, given the

limited number of industrial and commercial sites producing and processing talc in Canada, talc exposure from ambient air is not expected to be significant.

Talc is insoluble in water (Table 3-1) and is expected to settle out during water treatment; exposure to the general population from drinking water is not expected.

There is potential for oral exposure resulting from the use of talc as a food additive; however, exposure from these uses is expected to be minimal (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the use of talc as a component in food packaging materials is expected to be negligible (email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated February 27, 2018; unreferenced). Exposure from the oral route was not quantified because no critical health effects from the oral route of exposure have been identified. The JECFA has assigned an ADI of “not specified” for talc on the basis of low toxicity, and talc is “generally recognized as safe” as a food additive in the United States (JECFA 2006; U.S. FDA 2015).

6.2.2 Products available to consumers

Talc is present in approximately 8500 self-care products in Canada, including approximately 200 non-prescription drug products, approximately 2000 natural health products, and approximately 6500 cosmetic products. In addition, there are approximately 1300 prescription drugs containing talc. There is potential for oral exposure resulting from the use of self-care products and non-OTC drugs (including prescription, controlled substances, and ethical drugs) as a medicinal and non-medicinal ingredient containing talc. However, exposure from the oral route was not quantified as no critical health effects from the oral route of exposure have been identified.

There is the potential for dermal contact with talc from the use of self-care products. Systemic exposure resulting from dermal contact with talc is expected to be negligible as it is not expected that talc will be absorbed on the basis of its physical-chemical characteristics as an insoluble solid particle. In addition, a dermal health effect endpoint has not been identified for talc.

Notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, the LNHPD (modified 2018), the Drug Product Database (DPD), voluntary information submitted to Environment and Climate Change Canada and Health Canada (ECCC, HC 2017), publicly available databases and websites (e.g., Household Products Database 1993-; CPCat 2014; CPID 2017), and material safety and technical datasheets were used to identify products where there is: (a) the potential for inhalation of insoluble respirable talc, and (b) the potential exposure to the perineal region. These products and associated exposures are presented below.

No inhalation or perineal exposures were identified with respect to the major commercial or industrial uses of talc in paper, plastics, ceramics, and putties.

Inhalation exposure

For inhalation exposure, potential exposures were focused on products that were formulated as loose powders and were available to consumers, which included approximately 400 self-care products (primarily cosmetics). Products formulated as pressed powders, which comprise the majority of cosmetics containing talc (approximately 4000 products) were not identified as a potential source of exposure of concern because the formation of a “dust cloud” available for inhalation is not expected during the use of these products. Available information of interest were self-care products marketed as cosmetics, NHPs, or non-prescription drugs that are intended for application to the body, face, feet, buttocks (babies), and hair (e.g., dry hair shampoo). Concentrations of talc range from less than 10 to 100 % in these types of products.

In order to determine if talc loose-powder self-care products contain respirable particles, Health Canada measured the particle size distribution of three products (one baby powder and two adult body powder products) containing high concentrations of talc (>90%) available in Canada (Rasmussen 2017). Using an Aerodynamic Particle Sizer, the particle size distribution for the three products ranged from < 0.5 µm to 8 µm, with median particle sizes ranging from 1.7 to 2 µm. Thus, all of the particles were within the inhalable range (< 10 µm), and the median particle size was within the respirable range (< 4 µm). Number concentrations measured using a scanning mobility particle sizer indicated that the proportion of nano-sized particles (<100 nm) was small (< 10 %) to negligible, depending on the product.

Several studies were conducted by the cosmetic industry in the 1970s to provide data required to assess the safety of talc powder products and generate air concentrations (Aylott et al. 1979; Russell et al. 1979). These studies demonstrated that during the use of face, baby, and adult powders, there are quantifiable concentrations of respirable talc particles available for inhalation exposure. In 1978, Aylott and colleagues determined mean respirable air concentrations of 0.48 to 1.9 mg/m^3 of talc (< 7 µm) over 5 minutes for loose face powder, adult dusting powder, baby dusting powder, and micronized adult dusting powder. That same year, concentrations of talc (< 10 µm) of 0.19 mg/m^3 and 2.03 mg/m^3, respectively, were determined near the infant breathing zone during a simulation of routine application of talcum powder during diapering, and in the breathing zone of adults during the application of talcum powder to their body (Russell et al. 1979). In both of these studies, the highest air concentrations were associated with the adult application of talcum powder to their bodies over infant diapering and application of loose facial powder. There are uncertainties with the calculated talc concentrations determined from these studies due to limitations in the collection and analysis of talc concentrations on the basis of the use of older equipment, older sampling methods, and older talc products.

In 2017, a study assessing the health risk from the use of cosmetic talc from historical products was published (Anderson et al. 2017). This study included examining historical talc products from the 1960s and 1970s to characterize airborne respirable dust concentrations during the use of these products. To quantify respirable talc concentrations in the breathing zone, Anderson and colleagues (2017) designed a study where 5 volunteers were asked to apply historical talc products as they typically would in a bathroom setting. Cyclone air sampling devices were attached to the breathing zone of each volunteer. Each exposure simulation consisted of 8 application events, at six-minute intervals, for a total sampling duration of 48 minutes. This study design ensured that the sample mass on the sampling filter was large enough for quantification and accuracy, but it was not expected that during the typical use of a talc body powder that individuals apply talc every six minutes over a 48-minute window. Average talc concentrations over the 48-minute exposure simulation were calculated using the total measured mass (from 8 applications over 48 minutes) and the air volume over the entire 48-minute sampling period. Respirable talc concentrations ranged from 0.26 to 5.03 mg/m^3, and the average was 1.46 mg/m^3. The average air concentration by subject ranged from 0.44 to 3.28 mg/m^3. Respirable talc concentrations were more variable between subjects than within subjects, suggesting that individual behaviour has a strong influence in airborne concentrations.

In 2018, Health Canada conducted a small study in order to measure the air concentrations of particles in the breathing zone of adult volunteer subjects while they were applying talc-containing self-care products (Rasmussen 2018). Continuous, direct-reading, personal breathing-zone monitors (positioned beside the nose) measured average particulate matter of aerodynamic diameter of 4 µm or less (PM_4) concentrations of 0.48 ± 0.18 mg/m^3 and 1.80 ± 0.82 mg/m^3 for volunteers applying body powder and loose face powder, respectively. Subjects repeated the application in triplicate. These average concentrations fall within the range of concentrations measured by Anderson and colleagues (2017). In this study, the application of loose face powder resulted in the highest average air concentration in the immediate vicinity of the nose.

Several exposure scenarios were derived to characterize inhalation exposure to talc particles from the use of self-care products; namely, the use of baby, body, face, and foot powders (loose formulations), and dry hair shampoo. Average air concentrations by subject from Anderson et al. 2017 were combined with the body and face replicates from Rasmussen 2018 to obtain an overall average air concentration of 1.36 ± 0.97 mg/m^3. This value was used to estimate adjusted air concentrations for self-care products based on the highest concentration of talc present in these products. The results are summarized in Table 6-2. The inputs for each of these scenarios are outlined in Appendix A.

Table 6-2. Inhalation exposure estimates to talc from self-care products available to consumers

Product type	Age group	Concentration in air per event (mg/m^3)[a]	Adjusted exposure concentration (mg/m^3)[b]
Baby powder 100% talc	Infant and Adult	1.36	0.0071
Body powder 100% talc	Adult	1.36	0.0047
Face powder 100% talc	Adult	1.36	0.0047
Foot powder 97% talc	Adult	1.32	0.0034
Dry hair shampoo 100% talc	Adult	1.36	0.0011

[a] Average measured air concentrations (Anderson et al. 2017, Rasmussen 2018) × the highest concentration of talc in product type.
[b] Refer to Appendix A for details.

Perineal exposure

Several types of self-care products have the potential to result in exposure to the perineal region. There are several baby and body powders (approximately 50 products) with concentrations of talc that range from 0.3 to 100 %. There has been a decline in popularity of the use of talc for feminine hygiene practices over time; of 6000 North American women, 19 % of women born between 1920 and 1940 reported applying talc directly to the perineal region, but only 3% of women born after 1975 reported the same (Narod 2016). Houghton and colleagues (2014) reported that in 2001, the proportion of U.S. women who were users of perineal talc was estimated at 40 %, down from 52 % during 1993 to 1998.

There is a small number of diaper or rash cream self-care products (less than 10) which contains low concentrations of talc as a non-medicinal ingredient (up to 0.5 %). Talc is permitted as a medicinal ingredient in diaper rash products at concentrations from 45 to 100 % (Health Canada 2007); however, there are no diaper rash products listed in the LNHPD containing talc as a medicinal ingredient (LNHPD [modified 2018]).

Additional self-care products that have the potential for perineal exposure (approximately 100 products) include antiperspirants and deodorants (e.g., genital antiperspirants), body wipes, bath bombs, and to a lesser extent (due to wash off or removal) other bath products (i.e., soap, shower gel) and products associated with hair removal (e.g., epilatory products). These products are formulated as gels, sprays, loose powders, and solid cakes, and range in concentration from less than 1% to 100% talc.

As indicated in Section 4, there is no evidence to suggest that talc is currently being used as a dry lubricant on condoms or medical examination gloves in Canada. At present, these are not considered to be sources of perineal exposure.

As a quantitative point of departure could not be derived from the available literature, perineal exposure from the use of self-care products was not quantified.

6.3 Characterization of risk to human health

Consistent with other international regulatory and advisory bodies (Danish EPA, U.S. EPA, MAK-Commission, U.S. FDA, and JECFA), no critical health effects were identified via the oral or dermal routes of exposure. As such, oral exposure to talc resulting from food intake and use of self-care products are not of concern.

Critical health effects have been identified following inhalation exposure to respirable talc particles. From the available toxicological studies, a NOAEC of 2 mg/m^3 from the NTP inhalation studies in mice and rats was identified in which non-cancer lung effects, with lung overload, were noted at the next highest concentration of 6 mg/m^3.

The average air concentration of talc following the use of a loose-powder self-care product (1.36 mg/m^3) provides a small margin of exposure (i.e., 1.5) to the NOAEC of 2 mg/m^3. However, the NOAEC is derived from a study with an exposure profile of 6 hours per day, 5 days per week, over 4 weeks, while the actual exposure scenarios from the use of self-care products are intermittent, occurring in minutes per day, daily, or weekly over many years. To address the differences in exposure between the NTP study and the actual use pattern, both the NOAEC and the talc air concentrations were adjusted to a continuous exposure scenario according to U.S. EPA guidance on inhalation risk assessment to more accurately characterize potential risk (U.S. EPA 1994, 2009). The NOAEC of 2 mg/m^3 is equivalent to an adjusted concentration of 0.36 mg/m^3, as noted in the Health Effects section. The NOAEC of 2 mg/m^3 was extracted from a 4-week inhalation study as a NOAEC for chronic exposure was not available. Episodic exposures from product use are expected to increase lung load due to the long alveolar clearance of talc. The adjusted air concentrations from the use of self-care products are presented in Table 6-3.

Table 6-3. Relevant exposure and hazard values for talc, and margins of exposure, for determination of risk

Exposure scenario	Adjusted air concentration, CA (mg/m^3)[a]	Adjusted critical-effect level (mg/m^3)	Critical health effect endpoint	MOE
Baby powder 100% talc	0.0071	NOAEC[adj]: 0.36	non-cancer lung effects	50

Body powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Face powder 100% talc	0.0047	NOAEC[adj]: 0.36	non-cancer lung effects	76
Foot powder 97% talc	0.0034	NOAEC[adj]: 0.36	non-cancer lung effects	106
Dry hair shampoo 100% talc	0.0011	NOAEC[adj]: 0.36	non-cancer lung effects	327

Abbreviations: adj, adjusted; CA, concentration in air per event; MOE, margin of exposure.
[a] From Anderson et al. (2017) and Rasmussen (2018), respectively, based on the highest concentration in products. For most of these product types, there is a wide range of talc concentrations (< 10 to 100 %).

The margins of exposure (MOEs) between the adjusted critical-effect level and the adjusted air concentrations range from 50 to 327 for self-care products. The MOEs for baby powder, body powder, face powder, and foot powder are considered potentially inadequate to account for uncertainties in the health effects (including a lack of a NOAEC from chronic studies) and exposure databases. The MOE for dry hair shampoo is considered adequate to address uncertainties in the health effects and exposure databases.

Based on available human data, ovarian cancer was also identified as a critical health effect for the perineal route of exposure to talc. There is the potential for perineal exposure to talc from the use of various self-care products (e.g., body powder, baby powder, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs). As noted in the Health Effects section, a point of departure cannot be derived for this health effect. Data from published meta-analyses of epidemiological studies indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer (Huncharek et al. 2003; Langseth et al. 2008; Terry et al. 2013; Berge et al. 2018; Penninkilampi and Eslick 2018; Taher et al. 2018). As noted by Narod (2016), “It is unlikely that the association between talc and ovarian cancer is due to confounding and so it is fair to say that if there is a statistically robust relationship between talc use and ovarian cancer it is likely to be causal.” Similarly, Penninkilampi and Eslick (2018) noted that “the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association.” Taher and colleagues (2018) noted that “consistent with previous evaluations by the International Agency for Research on Cancer (2010), and more recent and subsequent evaluations by individual investigators (Penninkilampi and Eslick 2018; Berge et al. 2018; Terry et al. 2013), the present comprehensive evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.”

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. Given that there is the potential for perineal exposure to talc from the use of various self-care products, a potential concern for human health has been identified.

6.4 Uncertainties in evaluation of risk to human health

The inhalation of talc has been associated with a variety of non-cancerous lung effects, commonly termed talcosis. Dose-response data for lung effects in humans is, for the most part, lacking, and the use of animal data to quantify risk due to talc inhalation is considered appropriate. Despite the lack of exposure quantification, there are numerous case reports, as well as worker studies, that have identified non-cancer health effects from inhalation of talc powders. There is some uncertainty regarding the extrapolation of the NOAEC identified in animal models exposed for 6 hours per day for a short duration (4 weeks) to long-term episodic human exposures. The true NOAEC for chronic exposure is likely substantially lower than 2 mg/m^3.

Some self-care products, in particular, some face powders, may contain a cover or another mechanism that would reduce the potential for the generation of a particle or dust cloud, or that would reduce the concentration of the dust cloud during use of the product. There is uncertainty as to which products, and the proportion of products on the market, that incorporate these exposure-mitigation measures.

There are limitations with the human epidemiological data. Potential sources of bias include selection bias due to low response rates or from limiting subjects, and exposure misclassification due to recall bias (Taher et al. 2018). Muscat and Huncharek (2008) also proposed that symptoms of ovarian cancer prior to diagnosis may increase the perineal use of talc and bias the results. However, Narod (2016) and Berge and colleagues (2018) put less emphasis on recall bias. In studies where the exposure is simple (e.g., never versus ever use), recall bias is unlikely to be an important source of bias (Narod 2016). The positive association is strongest for the serous histologic type (Berge et al. 2018; Taher et al. 2018); findings that the association may vary by histologic type detracts from the hypothesis of report bias, as this type of bias would likely operate for all histologic types (Berge el al. 2018).

Ovarian cancer, in general, is not well understood (National Academy of Sciences, Engineering, and Medicine 2016), and a comparable animal model is not available. Health Canada has identified self-care products with the potential for perineal exposure (e.g., baby powder, body powders, diaper and rash creams, genital antiperspirants and deodorants, body wipes, bath bombs); however, there is no indication exactly how the products are being used, the extent to which they would contribute to perineal exposure, and with what frequency and amount.

Talc use during diapering is a confounder that was not adequately accounted for in the epidemiological studies. It has not been determined whether the internal female genital

tract is exposed to talc dusts during infancy (Muscat and Huncharek 2008). As well, not all the available human studies are clear as to the formulations used for perineal applications. It is possible that the identified cancer incidences are specific to loose-powder formulations; however, there is inadequate information to attribute the cancer incidences to other formulation types (e.g., creams).

7. Conclusion

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from talc. It is proposed to conclude that talc does not meet the criteria under paragraphs 64(*a*) or (*b*) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that talc meets the criteria under paragraph 64(*c*) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that talc meets one of the criteria set out in section 64 of CEPA.

Talc is proposed to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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Appendix A. Inhalation exposure estimates

Table A-1. Estimated inhalation exposure concentrations from self-care products containing loose powder talc available to consumers

Scenario	Talc product conc.[a]	Study[b] conc. (mg/m^3)	CA[b] (mg/m^3)	ET[c] (hr/d)	EF[d] (d/yr)	ED[e] (yr)	EC adjusted (mg/m^3)[b]
Baby powder, infants	100 %	1.36	1.36	0.125	365	4	0.0071
Baby powder, adults	100 %	1.36	1.36	0.125	365	8	0.0071
Body powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Face powder, adults	100 %	1.36	1.36	0.083	365	58	0.0047
Foot powder, adults	97 %	1.36	1.32	0.083	274	58	0.0034
Dry hair shampoo, adults	100 %	1.36	1.36	0.083	84	58	0.0011

Abbreviations: Conc., concentration; CA, concentration in air per event; ET, exposure time; EF, exposure frequency; ED, exposure duration; EC, adjusted exposure concentration.

[a] Highest concentration of talc found per product type from notifications submitted under the *Cosmetic Regulations* to Health Canada for talc, DPD [modified 2018], email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; LNHPD [modified 2018], email from the Non-prescription and Natural Health Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, dated March 20, 2017, unreferenced; Fiume et al. 2015; Household Product Database 1993-; CPCat 2014; CPID 2017; SDS Search Tool 2016.

[b] Average by subject from Anderson et al. 2107 and Rasmussen 2018 (unpublished). CA = average study concentration × maximum talc concentration in product.

[c] ET is 5 minutes/application based on median time spent in the bathroom following a shower or bath (U.S. EPA 2011) × number of applications/day, whereby baby powder assumes 1.5 applications/day (CTFA 1983); the rest assume 1 application/day.

[d] EF is assumed to be daily for baby, body (U.S. EPA 2011) and face powder (Ficheux et al. 2015); foot powder 0.75 times/day or 274 times/year (Ficheux et al. 2015); dry hair shampoo 0.23 times/day or 84 times/year (Ficheux et al. 2015).

[e] Assumed infant wears diapers up to 4 years, adult exposure to baby powder from diapering children, 4 years per child and assume 2 children per family (Statistics Canada 2016), adult exposure for body powder, and foot powder (80 years lifetime, 12 years child).

[f] Adjusted exposure concentration is calculated as per Equation 8 in the U.S. EPA 2009 guidance document “Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual,” where EC = (CA × ET × EF × ED)/AT, and AT = averaging time, which is on the basis of ED × 365 days/year × 24 hours/day.